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EXPRESS MAIL LABEL NO. EV059538917US
DATE OF DEPOSIT: March 27, 2002

JC922 Rec'd PCT/PTO 27 MAR 2002

FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371		4239-62489
		U S APPLICATION NO (If known, see 37 C F R § 1 5) Not yet assigned 10/089485
INTERNATIONAL APPLICATION NO. PCT/US00/26689	INTERNATIONAL FILING DATE September 29, 2000	PRIORITY DATE CLAIMED October 12, 1999

TITLE OF INVENTION
FIBROBLAST GROWTH FACTOR-5 (FGF-5) IS A TUMOR ASSOCIATED T-CELL ANTIGEN

APPLICANT(S) FOR DO/EO/US
Hanada and Yang

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. § 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. § 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. § 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. § 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. § 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. § 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. § 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.
    ☒ Copies of References Cited.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§ 3.28 and 3.31 and the Recordal fee of $40.00 is included.
13. ☒ A **FIRST** preliminary amendment (marked-up version of amended specification).
    ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    ☒ Abstract on a separate page.
    ☒ Written Opinion.
    ☒ Preliminary Examination Report
    ☒ International Search Report.
    ☒ Sequence Listing (paper copy, disk, Statement in Compliance)

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SLR:kdn 03/27/02 4239-62489 107422.doc

EXPRESS MAIL LABEL NO. EV059538917US
DATE OF DEPOSIT: March 27, 2002

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U.S. APPLICATION NO (if known, see 37 C.F.R. §1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
Not yet assigned 10/076485	PCT/US00/26689	4239-62489

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)):

Neither International Preliminary Examination fee (37 C.F.R § 1.482) nor International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,040.00**

International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... **$890.00**

International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO as an International Searching Authority..... **$740.00**

International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... **$710.00**

International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... **$100.00**

	CALCULATIONS (PTO USE ONLY)
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ **890.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R § 1.492(e)).	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	41 - 20 =	21	x **$18.00**	$ **378.00**
Independent Claims	4 - 3 =	1	x **$84.00**	$ **84.00**
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$280.00**	$

TOTAL OF ABOVE CALCULATIONS =	$ **1352.00**
☐ Reduction of 1/2 for filing by small entity. Small entity status is claimed for this application.	$
SUBTOTAL =	$ **0.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 C.F.R. §§ 1.492(f)). +	$
TOTAL NATIONAL FEE =	$ **0.00**
Fee for recording the enclosed assignment (37 C.F.R. § 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). **$40.00** per property. +	$ **40.00**
TOTAL FEES ENCLOSED =	$ **1392.00**
REFUND →	$
CHARGE →	$

a. ☒ A check in the amount of $1392.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees that may be required, or credit any overpayment, to Deposit Account No. 02-4550. A duplicate copy of this sheet is enclosed.

d. ☒ Please return the enclosed postcard to confirm that the items listed above have been received.

NOTE: Where an appropriate time limit under 37 C.F.R. § 1.494 or § 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

KLARQUIST SPARKMAN, LLP
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SIGNATURE
Sheree Lynn Rybak, Ph.D.
NAME

47,913
REGISTRATION NUMBER

cc: Docketing
Client

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Hanada and Yang
Art Unit:
Application No.
Filed: Herewith
For: FIBROBLAST GROWTH FACTOR-5 (FGF-5) IS A TUMOR ASSOCIATED T-CELL ANTIGEN
Examiner: not yet assigned
Date: March 27, 2002

COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

PRELIMINARY AMENDMENT

In the specification, page 1, line 3 after the title, insert the following:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This is a § 371 national stage of PCT/US00/26689, filed September 29, 2000, which in turn claims the benefit of United States Provisional Application No. 60/157,103 filed October 2, 1999.--

REMARKS

By this amendment, the cross-reference to related applications was added to the specification. No claims were amended, added, or cancelled. Therefore, claims 1-41 are still pending.

Therefore, no new matter is added by this amendment.

If there are any questions regarding this amendment, please telephone the undersigned at the telephone number below.

Respectfully submitted,

KLARQUIST SPARKMAN, LLP

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Marked-up Version of Amended Specification
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)

In the specification, page 1, line 3 after the title:

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a § 371 national stage of PCT/US00/26689, filed September 29, 2000, which in turn claims the benefit of United States Provisional Application No. 60/157,103 filed October 2, 1999.

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PCT/US00/26689

10/089485

FIBROBLAST GROWTH FACTOR-5 (FGF-5) IS A TUMOR ASSOCIATED T-CELL ANTIGEN

FIELD

This disclosure relates to the treatment of a neoplasm expressing FGF-5, for example by stimulation of the immune response to treat a neoplasm, more specifically to the use of FGF-5 to stimulate a cytotoxic T cell response against a neoplasm expressing FGF-5.

BACKGROUND

The use of immunologic agents has been proposed as an alternative for anti-neoplastic chemotherapy. For example, the administration of purified antigens, alone or in combination with various adjuvants, can be used to stimulate the B cell response. In addition, the regulation of circulating growth factors has been proposed as another way to treat tumor progression (e.g., U.S. Patent 5,919,459). Adjuvant formulations have also been proposed which are designed to generally stimulate cytotoxic T cells (see, e.g., PCT/US98/18495).

The process by which T cells recognize and interact with other cells is complex and involves cell surface complexes on the other cells of peptides and molecules referred to as human leukocyte antigens (HLA) or major histocompatibiltiy complexes (MHC). The interaction of T cells and complexes of MHC with an antigen is restricted, requiring a specific T cell for a specific complex of MHC and peptide antigen. For example, particular antigens have been identified which are expressed on cell surfaces, which can lead to lysis of the tumor cells by specific cytotoxic T cells. Genes that encode the antigens found on the surface of the cancer are called tumor rejection antigen precursors (TRAP) molecules, and the peptides derived from these genes are referred to as tumor rejection antigens (TRA) or tumor associated antigens (TAA). However, only a few TAA molecules have been recognized, and these TAAs are only known to be present on a limited number of tumor types (see, e.g., U.S. Patent No. 5,939,526).

Class I MHC molecules (referred to as HLAs, in humans) are expressed on the surface of almost all nucleated cells. A peptide fragment from the TAA is presented in the groove of the HLA, on the surface of the tumor. This allows cytotoxic T lymphocytes (CTLs) to recognize and destroy the tumor cells. In general, CTL responses are not directed against all possible epitopes of the TAA. Rather, they are restricted to a few immunodominant determinants. Additional factors, mostly linked to a processing event, can also play a role in dictating which of the many potential epitopes will be presented to the CTL cells.

A large number of CTL clones directed against melanoma have been obtained. In several instances, the antigens recognized by these CTLs have been characterized (for review see Parmiani,

Eur. J. Cancer. 34:S42-S47, 1998; Kirkin *et al.*, *APMIS.* 106:665-79, 1998). In contrast, although several CTL clones directed against renal cell carcinoma (RCC) have been obtained, very little information is available on the TAAs recognized by these CTLs.

FGF-5 (initially termed FGF-3) is an oncogene-encoded glycoprotein bearing mitogenic activity for fibroblasts and endothelial cells. FGF-5 was previously isolated by transfecting a bladder carcinoma DNA into NIH-3T3 cells (Zhan *et al.*, *Mol Cell Biol.* 8:3487-95, 1988 and corresponding U.S. Patent Nos. 5,155,217 (referred to herein as the '217 patent) and 5,238,916 (referred to herein as the '916 patent)). Both the nucleotide and protein sequences were submitted to GenBank under Accession Nos. NM_004464 and NP_004455 in 1990, which are incorporated by reference, as are the '217 and '916 patents.

Several publications describe the expression of FGF-5 in tumor cell lines and tissue samples. FGF-5 is expressed in RCC (Yoshimura *et al.*, *Cancer Lett.* 103:91-7, 1996), as well as cancers of the bladder, liver and endometrium (Zhan *et al.*, *Mol Cell Biol.* 8:3487-95, 1988; the '916 and '217 patents; Yoshimura *et al.*, *Cancer Lett.* 103:91-7, 1996), pancreas (Kornmann *et al.*, *Oncogene*. 15:1417-24, 1997), gastric and esophageal adenocarcinomas (Altorki *et al.*, *Cancer*, 72:649-57, 1993), breast (Cullen *et al.*, *Cancer Res.* 51:4978-85, 1991) and malignant melanomas (Albino *et al.*, *Cancer Res.* 51:4815-20, 1991). However, whether FGF-5 expression in these tissues is correlated with the immunogenicity of FGF-5 was not determined. In addition, none of these publications disclose the use of FGF-5 as an immune target for cancer immunotherapies.

Expression of FGF-5 has been observed in several murine embryonic tissues (Goldfarb *et al.*, *Ann N Y Acad Sci*. 638:38-52, 1991; Haub and Goldfarb, *Development*. 112:397-406, 1991) and some normal adult tissues including pancreatic tissue (Kornmann *et al.*, *Oncogene*, 15:1417-24, 1997) human fibroblasts (Werner *et al.*, *Oncogene*. 6:2137-44, 1991; Albino *et al.*, 1991), skeletal muscle (Hannon *et al.*, *J. Cell Biol.* 132:1151-9, 1996 and Hughes *et al.*, *Neuron*, 10:369-77, 1993); and adult neurotrophic cells (Goldfarb *et al.*, *Ann N Y Acad Sci*. 638:38-52, 1991; Kitaoka *et al.*, *Invest. Ophthalmol. Vis. Sci.* 35:3189-98, 1994).

Renal cell carcinoma, although not a common malignancy, accounts for 3% of all adult cancers. Conventional therapies involving the systemic administration of both interleukin-2 (IL-2) and interferon α (IFN-α) result in the long-term remission in less than 20% of patients with metastatic RCC. In addition, this approach is associated with unwanted side effects. To date, no highly effective treatment for RCC is available, and the survival time of the patients is very short. It is therefore important to develop new treatment strategies for these patients.

Osband (U.S. Patent No. 5,192,537) discloses a method for treating RCC by *in vitro* incubation of a patient's T-cells with a tumor extract. The activated T-cells are then infused into

the patient to reduce or eliminate the tumor burden. Cimetidine is concurrently administered to inhibit the patient's suppressor cells, and augment the anti-tumor response.

The '217 and the '916 patents demonstrate that FGF-5 is expressed in bladder, liver, and endometrial cancers. In addition, these patents disclose a method for treating FGF-5 expressing cancers by the administration of *antibodies* which recognize FGF-5. Such antibodies are also disclosed as diagnostic agents. However, there is no disclosure of FGF-5 expression in RCC, or how FGF-5 might be used to treat cancers using the CTL-mediated immune response.

Gospodarowicz (WO 97/30155) teaches the administration of an FGF-5 nucleic acid sequence, lacking the signal sequence, to promote angiogenesis in patients suffering from myocardial ischemia. The signal sequence was deleted to remove the oncogenic potential of FGF-5. Also disclosed are techniques for delivery of FGF-5 nucleic acid sequences to cells.

Gaugler *et al.* (*Immunogenetics*. 44:323-30, 1996) and corresponding U.S. Patent No. 5,939,526 describe the identification of RAGE1, a Renal tumor AntiGEn recognized by autologous CTLs. RAGE1 was observed in 37% of RCC cell lines, but only in one of 57 renal cell carcinoma samples. RAGE1 was also expressed in other cancers including: sarcomas, infiltrating bladder carcinomas, and melanomas, but not expressed in normal tissues, except the retina. Gaugler *et al.* propose that immunization of cancer patients against RAGE1 antigens may be an effective cancer immunotherapy, but also disclose that only a small percentage of patients with RCC might benefit, due to the low frequency of expression of RAGE1 in RCC tumor samples (1 of 57). Techniques for immunizing subjects against TAA are disclosed in U.S. Patent No. 5,939,526, which is incorporated by reference.

Several TAAs recognized by CTLs have been identified in melanomas (for a review see Kirkin *et al.*, *APMIS*. 106:665-79, 1998). These antigens include: MAGE, BAGE, GAGE, PRAME, NY-ESO-1, gp 100, TRP-1, TRP- CDK4, MUM-1, and β-catenin, some of which have been tested for their ability to treat cancer *in vivo*. Although some antigens such as MAGE and PRAME should potentially be highly immunogenic, only a few patients have been identified who respond to these TAAs *in vivo*, indicating their genuinely low immunogenicity. These studies demonstrate that it is difficult to predict with any certainty which TAA will elicit a functional response *in vivo*. In addition, the need remains to identify alternative TAAs which might generate a greater response *in vivo*.

SUMMARY OF THE DISCLOSURE

The disclosure includes a method of treating a subject having a neoplasm expressing or over-expressing FGF-5. In one embodiment, the method involves modulating an immune response, such as increasing or decreasing an immune response, or modulating FGF-5 expression or activity,

such as increasing or decreasing FGF-5 expression or activity. In particular embodiments, the neoplasm expressing or over-expressing FGF-5 can be a carcinoma, such as an adenocarcinoma. In other embodiments, the carcinoma is a carcinoma of the prostate, breast, bladder, or pancreas, or RCC, all of which express or overexposes FGF-5. In specific embodiments, the neoplasm is a RCC.

In one embodiment, the method includes modulating an immune response sufficient to stimulate a cytotoxic T cell response to a cell of the FGF-5 expressing or over-expressing neoplasm. In a particular embodiment, the method includes administering a therapeutically effective amount of an agent that modulates an immune response, such as an FGF-5 polypeptide (including variants, polymorphisms, mutants, fusions, and fragments thereof), and immunoreactive sensitized T cells sensitized with FGF-5.

Alternative embodiments of the method include administering a therapeutically effective amount of an FGF-5 polypeptide that modulates an immune response to treat a subject having a neoplasm expressing or over-expressing FGF-5. In some disclosed embodiments, the FGF-5 polypeptide protein has the amino acid sequence shown in either SEQ ID NOS: 4, 6, 8, 10, 12, 16, 18, or 19 or amino acid sequences that differ from those specified in SEQ ID NOS: 4, 6, 8, 10, 12, 16, 18 or 19 by one or more conservative amino acid substitutions, or amino acid sequences having at least 70% sequence identity to those sequences, for example sequences that are at least 80%, 85%, 90%, 95% or even 98% or 99% identical. In addition to such variants that retain the ability to modulate an immune response, fragments of the sequences that have or retain such activity may be used. Such fragments may, for example, include at least 50%, 70%, 75%, 90% or 95% of the amino acid residues of the FGF-5 peptide sequence. In other embodiments, the sequence is no more than 15 or 20 contiguous residues in length, however longer sequences (such as up to 60 or more amino acids in length) are also included. Also included are fragments and variants of the 60 amino acid sequence of SEQ ID NO 19, which includes the HLA-A3 restricted epitope.

Although the polypeptide or fragment can be administered to a subject in a therapeutically effective amount sufficient to stimulate an immune response against cells of the tumor, alternative embodiments of the disclosure include administering a nucleic acid encoding the FGF-5 polypeptide, or the fragment, polymorphism, or variant thereof, sufficient to stimulate a cytotoxic T cell response. Alternatively, a vector can be administered to the subject which includes a nucleic acid sequence which encodes and expresses FGF-5, or a fragment, polymorphism, or variant thereof, sufficient to stimulate a cytotoxic T cell response to a cell of the neoplasm. In particular examples, the vector is a viral vector, such as a retroviral vector.

The FGF-5 polypeptide (including variants, polymorphisms, mutants, fusions, and fragments thereof) that modulates an immune response, can also be administered to the subject by

administering an effective amount of a host cell expressing a recombinant nucleic acid encoding FGF-5 (including variants, polymorphisms, mutants, fusions, and fragments thereof), sufficient to stimulate a cytotoxic T cell response to a cell of the neoplasm, and inhibit neoplastic proliferation. In some embodiments, the inhibition of neoplastic proliferation induces a regression of the tumor, for example as determined by radiographic or other laboratory evidence of disease (such as serologic tumor markers, such as FGF-5 specific binding agents). The T cell response is sufficient to stimulate the T cell to react with a cell of the tumor, such as RCC.

Alternatively, the subject can be treated by administering to the subject an effective, neoplasm-inhibiting amount of immunoreactive sensitized T cells, wherein the sensitized T cells are sensitized with FGF-5. The immunoreactive sensitized T cells may be autologous or heterologous.

In yet other embodiments, the method includes modulating FGF-5 expression or activity, for example by increasing or decreasing FGF-5 expression or activity. In a particular embodiment, the method includes administering a therapeutically effective amount of an agent that decreases FGF-5 expression or activity, such as an FGF-5 antisense molecule or FGF-5 specific binding agent. In another particular embodiment, the method includes administering a therapeutically effective amount of an agent that increases FGF-5 expression or activity, such as FGF-5 polypeptides and nucleic acids encoding FGF-5 polypeptides. FGF-5 antisense molecules hybridize to an RNA or a plus strand of an FGF-5 nucleic acid (including variants, polymorphisms, mutants, fusions, and fragments thereof) and inhibit FGF-5 expression and/or activity by a desired amount, such as by at least 20%, 50%, 70%, 80% or 90%. In some embodiments, the FGF-5 specific binding agent is capable of specifically binding to an FGF-5 polypeptide (including variants, polymorphisms, mutants, fusions, and fragments thereof). In particular examples, the specific binding agent is an antibody, such as polyclonal antibodies, monoclonal antibodies, and fragments of monoclonal antibodies.

In view of the discovery that at least certain fragments of FGF-5 are HLA-A3 restricted, the disclosure also includes administering the FGF-5 polypeptide, or a fragment or variant, to HLA-A3+ individuals. Individuals can be pre-screened to select HLA-A3+ individuals to whom to administer the FGF-5 polypeptide or fragment.

In yet another embodiment, a method is disclosed for stimulating a cytotoxic T cell response against a RCC, by contacting the T cell with a therapeutically effective amount of an FGF-5 polypeptide or a cell expressing FGF-5 sufficient to stimulate the T cell to react with a cell of the RCC.

Also disclosed herein are methods for detecting an enhanced susceptibility of a subject to disease associated with abnormal FGF-5 expression, such as increased FGF-5 expression, by detecting the presence of multiple copies of an FGF-5 and/or transcription factor gene, and/or an

increase in FGF-5 protein in cells of a subject, such as a human. The disease may be a tumor (such as a malignant tumor) in which FGF-5 is abnormally increased, such as RCC and carcinoma of the prostate, breast, bladder, and pancreas. In other embodiments, the disease is Hippel-Lindau disease, horseshoe kidneys, adult polycystic kidney disease, and acquired renal cystic disease. In certain embodiments, the presence of multiple copies of an FGF-5 and/or transcription factor(s) gene can be detected by incubating a nucleic acid, such as an oligonucleotide, with the nucleic acid of the cell under conditions such that the oligonucleotide will specifically hybridize to an FGF-5 and/or transcription factor(s) gene present in the nucleic acid to form an oligonucleotide:FGF-5 and/or transcription factor(s) gene complex, and then detecting an increase or decrease of oligonucleotide:FGF-5 and/or transcription factor(s) complexes, wherein the presence of said complexes indicates the presence of an FGF-5 and/or transcription factor(s) gene.

The present disclosure also provides methods for detecting the presence of FGF-5 protein in a cell by incubating a specific binding agent of the present disclosure with proteins of the cell under conditions such that the specific binding agent will specifically bind to an FGF-5 protein present in the cell to form a specific binding agent:FGF-5 protein complex, and detecting an increase or decrease (or quantity) of specific binding agent:FGF-5 protein complexes, including the presence of the FGF-5 protein, wherein an increase of the complexes relative to specific binding agent:FGF-5 protein complexes in a non-neoplastic cell indicates expression or overexpression of FGF-5, and an enhanced susceptibility of the subject to a disease of abnormal FGF-5 expression.

Also disclosed herein are methods for lysing a cell of an FGF-5 expressing neoplasm by enhancing an immune response against FGF-5 in the subject, sufficient to induce regression of the neoplasm. In one embodiment, the cell is characterized by increased expression of an FGF-5 protein, or the cell has increased FGF-5 expression or copy number, relative to FGF-5 expression in a same tissue type that is non-neoplastic. In certain embodiments, enhancing the immune response can be achieved by exposing the cell to a therapeutically effective amount of an FGF-5 polypeptide (including fragments, polymorphisms, fusions, and variants) disclosed herein. In other embodiments, enhancing the immune response can be achieved by administering a therapeutically effective amount of any FGF-5-expressing nucleic acid (including fragments, polymorphisms, fusions, and variants). In yet other embodiments, enhancing the immune response is achieved by administering a therapeutically effective amount of immuno-reactive sensitized T-cells wherein the sensitized T cells are sensitized with FGF-5.

The agents disclosed herein (such as those which modulate an immune response those which modulate FGF-5 expression or activity) can be administered prophylactically, prior to the development of a tumor, for example to persons at elevated risk of developing the tumor (such as persons with a family history of the tumor, and persons with Hippel-Lindau disease, horseshoe

kidneys, adult polycystic kidney disease, and acquired renal cystic disease). The agents can also be administered therapeutically, to a person who already has developed the tumor, for example a person who has undergone surgical resection of a RCC primary or metastatic lesion, or a person who is undergoing chemotherapy for treatment of the tumor. Alternatively, the agents can be administered as the sole therapy for the tumor. The agent(s) can be administered with pharmaceutically acceptable carrier. In addition, the agents can be administered in combination with other therapeutic treatments, such as other anti-neoplastic or anti-tumorigenic therapies. Such agents can be administered concurrently or sequentially, with the other therapies.

The foregoing and other objects, features, and advantages disclosed herein will become more apparent from the following detailed description of several embodiments which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a bar graph and table showing the analysis of the reactivity of Clone 2 CTL using a panel of tumor lines, and demonstrating HLA-A3 restriction of the CTL response to FGF-5. The HLA type of each tumor is shown on the right.

FIGS. 2A and 2B are bar graphs showing the effect of various anti-HLA monoclonal antibodies on the recognition of tumors by (A) an HLA-A2 restricted CTL or (B) Clone 2 CTL.

FIG. 3A is a bar graph illustrating the recognition of three cDNA clones in a HLA-A3 restricted manner by Clone 2 CTL.

FIG. 3B is a schematic drawing showing the alignment of the three tumor-derived FGF-5 clones. The nucleotide changes (inside each bar), their positions (under 10E4-1), and any resulting amino acid changes (above each bar) are shown.

FIG. 4 is a graphical representation showing the normalization of FGF-5:β-actin copy number in normal adult tissues.

FIG. 5 is a graphical representation showing the normalization of FGF-5 copy number by β-actin copy number (filled bars) and the recognition by the CTL as represented by IFN-γ concentration (x), in each cell line. In the insert graph, the correlation between FGF-5 expression and recognition by the CTL ($p < 0.0001$) is shown.

FIG. 6 is a sequence listing of an FGF-5 ORF-1 and ORF-2 from U.S. Patent No. 5,238,916, which is incorporated by reference.

FIG. 7 is a graphical representation showing FGF-5 peptides that are immunogenic (unfilled bars) and are non-immunogenic (filled bars). The numbers below each bar represent the nucleotide number (upper numbers), and amino acid number (lower numbers) of the FGF-5 sequences shown in SEQ ID NO: 17.

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand.

SEQ ID NO 1 shows a DNA sequence for a first open reading frame of MUSFGF-5A.

SEQ ID NO 2 shows an amino acid sequence for a first open reading frame of MUSFGF-5A.

SEQ ID NO 3 shows a DNA sequence for a second open reading frame of MUSFGF-5A.

SEQ ID NO 4 shows an amino acid sequence for a second open reading frame of MUSFGF-5A.

SEQ ID NO 5 shows a cDNA sequence for a variation of IA3-1.

SEQ ID NO 6 shows an amino acid sequence for a variation of IA3-1.

SEQ ID NO 7 shows a cDNA sequence for another variation of IA3-1.

SEQ ID NO 8 shows an amino acid sequence for another variation of IA3-1.

SEQ ID NO 9 shows a cDNA sequence for a variation of 6A4-1.

SEQ ID NO 10 shows an amino acid sequence for a variation of 6A4-1.

SEQ ID NO 11 shows a cDNA sequence for another variation of 6A4-1.

SEQ ID NO 12 shows the amino acid sequence for another variation of 6A4-1.

SEQ ID NO 13 shows a cDNA sequence for a variation of 10E4-1.

SEQ ID NO 14 shows a cDNA sequence for another variation of 10E4-1.

SEQ ID NO 15 shows a full-length cDNA sequence of construct 10E4-1 with ORF-1.

SEQ ID NO 16 shows an amino acid sequence of ORF-1 from construct 10E4-1.

SEQ ID NO 17 shows a full-length cDNA sequence of construct 10E4-1 with ORF-2.

SEQ ID NO 18 shows an amino acid sequence of ORF-2 from construct 10E4-1.

SEQ ID NO 19 shows a 60 amino acid sequence which contains the FGF-5 epitope for HLA-A3+ individuals.

SEQ ID NO 20 and 21 show nucleic acid sequences of forward primers that can be used to RT-PCR FGF-5.

SEQ ID NO 22 and 23 show nucleic acid sequences of reverse primers that can be used to RT-PCR FGF-5.

SEQ ID NO 24 and 25 show nucleic acid sequences of forward and reverse primers, respectively, that can be used to clone the HLA-A3 gene from autologous 1764 RCC by RT-PCR.

DETAILED DESCRIPTION OF SEVERAL EMBODIMENTS

The following definitions and methods are provided to better define the present disclosure and to guide those of ordinary skill in the art in the practice of the present disclosure. It must be noted that as used herein and in the appended claims, the singular forms "a" or "an" or "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" includes a plurality of such proteins and reference to "the antibody" includes reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs.

Abbreviations and Definitions

CTL	cytotoxic T lymphocyte
FGF-5	fibroblast growth factor-5
RCC	renal cell carcinoma
RT	room temperature
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TAA	tumor-associated antigen
TCR	T-cell receptor
TILs	tumor infiltrating lymphocytes

Antineoplastic agent: A drug or biologic that inhibits the proliferation of neoplastic cells, for example arresting their growth or causing the regression of a tumor. Examples include alkylating agents (such a vincristine, vinblastine or taxol), anthracycline antibiotics such as daunorubicin and doxorubicin, hormonal therapies such as tamoxifen, and miscellaneous agents such as cis-diamminedichloroplatimun (II), and hydroxyurea. Antineoplastic agents also include biologics, such as IL-2 and alpha-interferon, and immunotherapy, for example with bacille Calmette-Guerin (BCG). Protocols for administration of such agents are known in the art, and examples can be found in Goodman and Gilman, The Pharmacological Basis of Therapeutics, 17th edition, section XIII.

Antisense molecules or antisense oligonucleotides: Nucleic acid molecules that are specifically hybridizable or specifically complementary to either RNA or the plus strand of DNA (Weintraub, *Scientific American* 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids

interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double stranded. In one embodiment, the antisense oligomer is about 15 nucleotides, which are easily synthesized. The use of antisense molecules to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal. Biochem.* 172:289, 1988).

Therapeutically effective antisense molecules are characterized by their ability to inhibit the expression of FGF-5. Complete inhibition is not necessary for therapeutic effectiveness, some oligonucleotides will be capable of inhibiting the expression of FGF-5 by at least 15%, 30%, 40%, 50%, 60%, 70%, 80% or 90%.

Therapeutically effective antisense molecules are additionally characterized by being sufficiently complementary to FGF-5 encoding nucleic acid sequences. As described below, sufficient complementary means that the therapeutically effective oligonucleotide or oligonucleotide analog can specifically disrupt the expression of FGF-5, and not significantly alter the expression of genes other than FGF-5.

Cancer: Malignant neoplasm that has undergone characteristic anaplasia with loss of differentiation, increased rate of growth, invasion of surrounding tissue, and is capable of metastasis.

cDNA (complementary DNA): A piece of DNA lacking internal, non-coding segments (introns) and regulatory sequences which determine transcription. cDNA is synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

Chemical synthesis: The artificial means by which one can make a protein or peptide, for example as described in EXAMPLE 17.

Deletion: The removal of a sequence of DNA, the regions on either side being joined together.

DNA: Deoxyribonucleic acid. DNA is a long chain polymer which comprises the genetic material of most living organisms (some viruses have genes comprising ribonucleic acid, RNA). The repeating units in DNA polymers are four different nucleotides, each of which comprises one of the four bases, adenine, guanine, cytosine and thymine bound to a deoxyribose sugar to which a phosphate group is attached. Triplets of nucleotides, referred to as codons, in DNA molecules code for amino acid in a polypeptide. The term codon is also used for the corresponding (and complementary) sequences of three nucleotides in the mRNA into which the DNA sequence is transcribed.

FGF-5 cDNA: A FGF-5 cDNA is functionally defined as cDNA molecule which encodes a protein having the ability to modulate an immune response, and includes fragments, variants, and polymorphisms of an FGF-5 cDNA that retain the ability to modulate an immune response. The FGF-5 cDNA can be derived by reverse transcription from the mRNA encoded by a FGF-5 gene

and lacks internal non-coding segments and transcription regulatory sequences present in the FGF-5 gene.

FGF-5 fusion protein: A fusion protein comprising a FGF-5 protein (or variants, polymorphisms, mutants, or fragments thereof) linked to other amino acid sequences that do not inhibit the desired activity of FGF-5, for example an immunogenic activity. The other amino acid sequences may be, for example, no more than 10, 20, 30, or 50 amino acid residues in length..

FGF-5 Gene: A gene which encodes an FGF-5 protein having the ability to modulate an immune response. The definition of a FGF-5 gene includes the various sequence polymorphisms and allelic variations that may exist within a population, or in other species.

FGF-5 Protein or FGF-5 Polypeptide: A protein encoded by a FGF-5 gene or cDNA, as well as fragments, variants, and polymorphisms that retain the ability to modulate an immune response. This protein may be functionally characterized by its ability to modulate an immune response as described herein. FGF-5 proteins include the full-length FGF-5 transcript (SEQ ID NOS: 4 and 18), as well as shorter peptides (for example SEQ ID NOS: 6, 8, 10, 12, and 19) and fusion proteins which retain the ability to modulate an immune response.

FGF-5 RNA: The RNA transcribed from a FGF-5 gene.

Isolated: An "isolated" biological component (such as a nucleic acid, peptide or protein) has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids, peptides and proteins which have been "isolated" thus include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids. The method of the present disclosure can include administration of isolated FGF-5 to a subject.

Malignant: Cells which have the properties of anaplasia invasion and metastasis.

Mammal: This term includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

Mimetic: A molecule (such as an organic chemical compound) that mimics the activity of a protein, such as the ability to modulate an immune response. Peptidomimetic and organomimetic embodiments are within the scope of this term, wherein the three-dimensional arrangement of the chemical constituents of such peptido- and organomimetics mimic the three-dimensional arrangement of the peptide backbone and component amino acid sidechains in the peptide, resulting in such peptido- and organomimetics of the peptides having the ability to stimulate a CTL response against a tumor that is expressing or overexpressing FGF-5. For computer modeling applications, a pharmacophore is an idealized, three-dimensional definition of the structural requirements for

biological activity. Peptido- and organomimetics can be designed to fit each pharmacophore with current computer modeling software (using computer assisted drug design or CADD). See Walters, "Computer-Assisted Modeling of Drugs", in Klegerman & Groves, eds., 1993, Pharmaceutical Biotechnology, Interpharm Press: Buffalo Grove, IL, pp. 165-174 and Principles of Pharmacology (ed. Munson, 1995), chapter 102 for a description of techniques used in computer assisted drug design. EXAMPLE 16 describes other methods which can be used to generate mimetics.

Modulating an Immune Response: Includes the ability to increase or decrease an immune response in a subject, such as the ability to stimulate a CTL immune response, such as an HLA-A3-restricted CTL response, against FGF-5 expressing or over-expressing tumors, by a desired amount.

Agents that modulate an immune reponse include, but are not limited to: FGF-5 polypeptides (including fragments, variants, fusion proteins, and polymorphisms thereof), FGF-5 nucleic acid molecules encoding FGF-5- polpeptides, FGF-5 specific binding agent, FGF-5 antisense molecules, and immunoreactive sensitized T cells sensitized with FGF-5.

Modulating FGF-5 expression or activity: Includes increasing or decreasing FGF-5 expression or activity. In one embodiment, modulating FGF-5 expression or activity includes administering a therapeutically effective amount of an agent that decreases FGF-5 expression or activity, such as an FGF-5 antisense molecule or FGF-5 specific binding agent. In yet another particular embodiment, modulating FGF-5 expression or activity includes administering a therapeutically effective amount of an agent that increases FGF-5 expression or activity, such as the FGF-5 polypeptides and nucleic acids encoding FGF-5 polypeptides disclosed herein.

Mutant FGF-5 gene: A mutant form of a FGF-5 gene which in some embodiments is associated with disease, for example a carcinoma, such as an adenocarcinoma, RCC, breast cancer, and prostate cancer. In other embodiments, the disease is an FGF-5-expressing tumor, for example RCC, breast, and prostate cancers.

Mutant FGF-5 protein: A protein encoded by a mutant FGF-5 gene.

Mutant FGF-5 RNA: An RNA transcribed from a mutant FGF-5 gene.

Neoplasm: Abnormal growth of cells.

Normal cells: Non-tumor, non-malignant cells.

Oligonucleotide: A linear polynucleotide sequence of up to about 200 nucleotide bases in length, for example a polynucleotide (such as DNA or RNA) which is at least 6 nucleotides, for example at least 15, 25, 50, 100 or even 200 nucleotides long.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the

second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

ORF (open reading frame): A series of nucleotide triplets (codons) coding for amino acids without any internal termination codons. These sequences are usually translatable into a peptide.

Ortholog: Two nucleotide sequences are orthologs of each other if they share a common ancestral sequence, and diverged when a species carrying that ancestral sequence split into two species. Orthologous sequences are also homologous sequences.

PCR (polymerase chain reaction): Describes a technique in which cycles of denaturation, annealing with primer, and then extension with DNA polymerase are used to amplify the number of copies of a target DNA sequence.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers useful herein are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the DNA, RNA, proteins, and antibodies herein disclosed. Embodiments of the disclosure comprising medicaments can be prepared with conventional pharmaceutically acceptable carriers, adjuvants and counterions as would be known to those of skill in the art.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol, ethanol, sesame oil, combinations thereof, or the like, as a vehicle. The medium may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. The carrier and composition can be sterile, and the formulation suits the mode of administration. For solid compositions (e.g., powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, sodium saccharine, cellulose, magnesium carbonate, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Further embodiments are provided in EXAMPLE 18.

Polynucleotide: A linear nucleic acid sequence of any length. Therefore, a polynucleotide includes molecules which are 15, 50, 100, 200 (oligonucleotides) and also nucleotides as long as a full length cDNA. In particular embodiments, the polynucleotides are no longer than 15, 50, 100, 120, 180, or 200 nucleotides in length.

Probes and primers: Nucleic acid probes and primers may readily be prepared based on the amino acid sequences provided herein. A probe comprises an isolated nucleic acid attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. Methods for labeling and guidance in the choice of labels appropriate for various purposes are discussed, e.g., in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989) and Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Intersciences (1987).

Primers are short nucleic acids, such as DNA oligonucleotides 15 nucleotides or more in length. Primers may be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by PCR or other nucleic-acid amplification methods known in the art.

Methods for preparing and using probes and primers are described, for example, in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989), Ausubel et al., 1987, and Innis et al., PCR Protocols, A Guide to Methods and Applications,1990, Innis et al. (eds.), 21-27, Academic Press, Inc., San Diego, California. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, MA).

Promoter: An array of nucleic acid control sequences which direct transcription of a nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified peptide preparation is one in which the peptide or

protein is more enriched than the peptide or protein is in its natural environment within a cell. Preferably, a preparation is purified such that the protein or peptide represents at least 50% of the total peptide or protein content of the preparation. The method disclosed herein can include administration of purified FGF-5 to a subject to provoke a CTL response against a tumor that is expressing or overexpressing FGF-5.

Recombinant: A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques.

Sample: Includes biological samples containing genomic DNA, RNA, or protein obtained from body cells, such as those present in peripheral blood, urine, saliva, tissue biopsy, surgical specimen, amniocentesis samples and autopsy material.

Sequence identity: The similarity between two nucleic acid sequences, or two amino acid sequences, is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are. Homologs or orthologs of nucleic acid or amino acid sequences will possess a relatively high degree of sequence identity when aligned using standard methods. This homology will be more significant when the orthologous proteins or cDNAs are derived from species which are more closely related (e.g., human and chimpanzee sequences), compared to species more distantly related (e.g., human and *C. elegans* sequences). Typically, FGF-5 orthologs are at least 50% identical at the nucleotide level and at least 50% identical at the amino acid level when comparing orthologus sequences.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith & Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman & Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444, 1988; Higgins & Sharp, *Gene*, 73:237-44, 1988; Higgins & Sharp, *CABIOS* 5:151-3, 1989; Corpet et al., *Nuc. Acids Res.* 16:10881-90, 1988; Huang *et al. Computer Appls. in the Biosciences* 8, 155-65, 1992; and Pearson *et al.*, *Meth. Mol. Bio.* 24:307-31, 1994. Altschul *et al*,. *J. Mol. Biol.* 215:403-10, 1990, presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, *J. Mol. Biol.* 215:403-10, 1990) is available from several sources, including the National Center for Biological Information (NCBI, National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD

20894) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. Additional information can be found at the NCBI web site.

Homologs of an FGF-5 protein are typically characterized by possession of at least 70%, 80%, 85%, 90%, 95%, 98% or 99%% sequence identity counted over full-length alignment with the amino acid sequence of FGF-5 using the NCBI Blast 2.0, gapped blastp set to default parameters. Queries searched with the blastn program are filtered with DUST (Hancock, and Armstrong, 1994, *Comput. Appl. Biosci.* 10:67-70). Other programs use SEG. Alternatively, one may manually align the sequences and count the number of identical amino acids. This number divided by the total number of amino acids in the reference sequence multiplied by 100 results in the percent identity.

For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequence will show increasing percentage identities when assessed by this method, such as at least 70%, 80%, 85%, 90%, 95%, 98%, 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs will typically possess at least 75% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85%, 90%, 95%, 98% or 99% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are described at the NCBI web site.

One of ordinary skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided. Provided herein are the peptide homologs described above, as well as nucleic acid molecules that encode such homologs.

An alternative indication that two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions. Stringent conditions are sequence-dependent and are different under different environmental parameters. Generally, stringent conditions are selected to be about 5 C to 20 C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence remains hybridized to a perfectly matched probe or complementary strand. Conditions for nucleic acid hybridization and calculation of stringencies can be found in Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, CSHL, New York, 1988) and Tijssen (*Laboratory Techniques in Biochemistry and Molecular Biology--*

Hybridization with Nucleic Acid Probes Part I, Chapter 2, Elsevier, New York, 1993). Nucleic acid molecules that hybridize under stringent conditions to an FGF-5 gene sequence will typically hybridize to a probe based on either an entire FGF-5 gene or selected portions of the gene under wash conditions of 2x SSC at 50 C. A more detailed discussion of hybridization conditions is presented in EXAMPLE 7.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences, due to the degeneracy of the genetic code. It is understood that changes in nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid molecules that all encode substantially the same protein.

Such homologous peptides may, for example, possess at least 70%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity determined by this method. When less than the entire sequence is being compared for sequence identity, homologs may, for example, possess at least 70%, 75%, 85% 90%, 95%, 98% or 99% sequence identity over short windows of 10-20 amino acids. Methods for determining sequence identity over such short windows can be found at the NCBI web site. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs or other variants could be obtained that fall outside of the ranges provided.

The disclosure provides not only the peptide homologs that are described above, but also nucleic acid molecules that encode such homologs.

An alternative (and not necessarily cumulative) indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences, due to the degeneracy of the genetic code. It is understood that changes in nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

Specific binding agent: An agent that binds substantially only to a defined target. As used herein, the term "FGF-5 specific binding agent" includes anti-FGF-5 peptide antibodies and other agents that bind substantially only to an FGF-5 peptide. The antibodies may be monoclonal or polyclonal antibodies that are specific for an FGF-5 peptide, as well as immunologically effective portions ("fragments") thereof. In one embodiment, the antibodies used herein are monoclonal antibodies (or immunologically effective portions thereof) and may also be humanized monoclonal antibodies (or immunologically effective portions thereof). Immunologically effective portions of monoclonal antibodies include Fab, Fab', $F(ab')_2$, Fabc and Fv portions (for a review, see Better and Horowitz, *Methods. Enzymol.* 178:476-96, 1989). Anti-inhibitory peptide antibodies

may also be produced using standard procedures described in a number of texts, including *Antibodies, A Laboratory Manual* by Harlow and Lane, Cold Spring Harbor Laboratory (1988).

The determination that a particular agent binds substantially only to a FGF-5 peptide may readily be made by using or adapting routine procedures. One suitable *in vitro* assay makes use of the Western blotting procedure (described in many standard texts, including *Antibodies, A Laboratory Manual* by Harlow and Lane). Western blotting may be used to determine that a given FGF-5 peptide binding agent, such as an anti-FGF-5 peptide monoclonal antibody, binds substantially only to a FGF-5 protein. The specific binding agents disclosed herein may be used to modulate FGF-5 activity, for example to cause regression of an FGF-5 expressing or over-expressing neoplasm. In addition, specific binding agents of disclosed herein may be used for diagnostic purposes, for example to determine if a subject has an enhanced susceptibility to develop a disease associated with FGF-5 expression or over-expression, or to monitor a subject's progress during therapy to treat an FGF-5 expressing or over-expressing neoplasm

Specifically hybridizable and specifically complementary: Terms which indicate a sufficient degree of complementarity such that stable and specific binding occurs between the oligonucleotide (or its analog) and the DNA or RNA target. The oligonucleotide or oligonucleotide analog need not be 100% complementary to its target sequence to be specifically hybridizable. An oligonucleotide or analog is specifically hybridizable when binding of the oligonucleotide or analog to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide or analog to non-target sequences under conditions in which specific binding is desired, for example under physiological conditions in the case of *in vivo* assays. Such binding is referred to as "specific hybridization." See EXAMPLE 7 for examples of hybridization conditions.

Subject: Living multicellular vertebrate organisms, a category which includes, both human and veterinary subjects for example, mammals, birds and primates.

Sufficient complementarity: When used, indicates that a sufficient number of base pairs exist between the oligonucleotide and the target sequence to achieve detectable binding, and disrupt expression of gene products (such as FGF-5). When expressed or measured by percentage of base pairs formed, the percentage complementarity that fulfills this goal can range from as little as about 50% complementarity to full, (100%) complementary. In general, sufficient complementarity is at least about 50%. In one embodiment, sufficient complementarity is at least about 75% complementarity. In another embodiment, sufficient complementarity is about 90% or about 95% complementarity. In yet another embodiment, sufficient complementarity is about 98% or 100% complementarity.

A thorough treatment of the qualitative and quantitative considerations involved in establishing binding conditions that allow one skilled in the art to design appropriate oligonucleotides for use under the desired conditions is provided by Beltz *et al. Methods Enzymol* 100:266-285, 1983, and by Sambrook et al. (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

Target sequence: A portion of single-stranded DNA (ssDNA), double-stranded DNA (dsDNA) or RNA that upon hybridization to an therapeutically effective oligonucleotide or oligonucleotide analog results in the inhibition of VIAF expression. Either an antisense or a sense molecule can be used to target a portion of dsDNA, since both will interfere with the expression of that portion of the dsDNA. The antisense molecule can bind to the plus strand, and the sense molecule can bind to the minus strand. Thus, target sequences can be ssDNA, dsDNA, and RNA.

An oligonucleotide "binds" or "stably binds" to a target nucleic acid if a sufficient amount of the oligonucleotide forms base pairs or is hybridized to its target nucleic acid, to permit detection of that binding. Binding can be detected by either physical or functional properties of the target:oligonucleotide complex. Binding between a target and an oligonucleotide can be detected by any procedure known to one skilled in the art, including both functional and physical binding assays. Binding may be detected functionally by determining whether binding has an observable effect upon a biosynthetic process such as expression of a gene, DNA replication, transcription, translation and the like.

Physical methods of detecting the binding of complementary strands of DNA or RNA are well known in the art, and include such methods as DNase I or chemical footprinting, gel shift and affinity cleavage assays, Northern blotting, dot blotting and light absorption detection procedures. For example, one method that is widely used, because it is so simple and reliable, involves observing a change in light absorption of a solution containing an oligonucleotide (or an analog) and a target nucleic acid at 220 to 300 nm as the temperature is slowly increased. If the oligonucleotide or analog has bound to its target, there is a sudden increase in absorption at a characteristic temperature as the oligonucleotide (or analog) and target disassociate or melt.

The binding between an oligomer and its target nucleic acid is frequently characterized by the temperature (T_m) at which 50% of the oligomer is melted from its target. A higher (T_m) means a stronger or more stable complex relative to a complex with a lower (T_m).

Therapeutically Effective Amount: The preparations disclosed herein are administered in therapeutically effective amounts. An effective amount is that amount of a pharmaceutical preparation that alone, or together with further doses, stimulates the desired response. In the case of treating cancer, the desired response is inhibiting or stopping the progression of the cancer, or inducing regression of the disease. Treatment may involve only slowing the progression of the

disease temporarily, although more preferably, it involves halting or reversing the progression of the disease permanently.

The therapeutically effective amount also includes a quantity of FGF-5 protein, fusion protein, FGF-5 antisense molecule, FGF-5 specific binding agent, and/or autologous CTLs specific to FGF-5, sufficient to achieve a desired effect in a subject being treated. For instance, this can be the amount necessary to improve signs and/or symptoms a disease such as cancer, for example by modulating, for example increasing a CTL response against a tumor expressing or overexpressing FGF-5.

An effective amount of FGF-5 protein, FGF-5 antisense molecule, FGF-5 specific binding agent, and/or autologous CTLs specific to FGF-5, may be administered in a single dose, or in several doses, for example daily, during a course of treatment. However, the effective amount of will be dependent on the source applied (i.e. FGF-5 isolated from a cellular extract versus a chemically synthesized and purified FGF-5, or a variant or fragment that may not retain full FGF-5 activity), the subject being treated, the severity and type of the condition being treated, and the manner of administration. For example, a therapeutically effective amount of FGF-5 protein, FGF-5 antisense molecule, FGF-5 specific binding agent, and/or autologous CTLs specific to FGF-5 can vary from about 0.01 mg/kg body weight to about 1 g/kg body weight.

The methods disclosed herein have equal application in medical and veterinary settings. Therefore, the general term "subject being treated" is understood to include all animals (e.g. humans, apes, dogs, cats, horses, and cows) that require modulation of a CTL response against a tumor that is expressing or overexpressing FGF-5.

Therapeutically effective dose: A dose sufficient to stimulate a CTL response to a tumor expressing or over-expressing FGF-5, resulting in a regression of a pathological condition, or which is capable of relieving signs or symptoms caused by the condition, such as regression of the tumor and/or lysis of the cells of the tumor.

Transformed: A transformed cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques. As used herein, the term transformation encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

Transgenic Cell: Transformed cells which contain foreign, non-native DNA.

Tumor: A neoplasm

Variants or fragments: The production of FGF-5 protein can be accomplished in a variety of ways (for example see EXAMPLE 8). DNA sequences which encode for the protein, or a fragment or variant of the protein, can be engineered such that they allow the protein to be

expressed in eukaryotic cells, bacteria, insects, and/or plants. In order to accomplish this expression, the DNA sequence can be altered and operably linked to other regulatory sequences. The final product, which contains the regulatory sequences and the therapeutic protein, is referred to as a vector. This vector can then be introduced into the eukaryotic cells, bacteria, insect, and/or plant. Once inside the cell the vector allows the protein to be produced.

One of ordinary skill in the art will appreciate that the DNA can be altered in numerous ways without affecting the activity of the encoded protein. For example, PCR may be used to produce variations in the DNA sequence which encodes FGF-5. Such variants may be variants that are optimized for codon preference in a host cell that is to be used to express the protein, or other sequence changes that facilitate expression.

Two types of cDNA sequence variant may be produced. In the first type, the variation in the cDNA sequence is not manifested as a change in the amino acid sequence of the encoded polypeptide. These silent variations are simply a reflection of the degeneracy of the genetic code. In the second type, the cDNA sequence variation does result in a change in the amino acid sequence of the encoded protein. In such cases, the variant cDNA sequence produces a variant polypeptide sequence. In order to optimize preservation of the functional and immunologic identity of the encoded polypeptide, any such amino acid substitutions may be conservative. Conservative substitutions replace one amino acid with another amino acid that is similar in size, hydrophobicity, etc.

Variations in the cDNA sequence that result in amino acid changes, whether conservative or not, are minimized to enhance preservation of the functional and immunologic identity of the encoded protein. The immunologic identity of the protein may be assessed by determining whether it is recognized by an antibody to FGF-5; a variant that is recognized by such an antibody is immunologically conserved. In particular embodiments, any cDNA sequence variant will introduce no more than 20, for example fewer than 10 amino acid substitutions into the encoded polypeptide. Variant amino acid sequences can, for example, be 70%, 80%, 90% or even 95% identical to the native amino acid sequence.

Conserved residues in the same or similar proteins from different species can also provide guidance about possible locations for making substitutions in the sequence. A residue which is highly conserved across several species is more likely to be important to the function of the protein than a residue that is less conserved across several species.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art.

Additional definitions of terms commonly used in molecular genetics can be found in Benjamin Lewin, *Genes V* published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

To identify TAA, tumor infiltrating lymphocytes (TIL) were obtained from a metastatic pulmonary lesion from a renal cell carcinoma patient who demonstrated a mixed spontaneous regression of lesions following nephrectomy. These TIL were multiply restimulated with an autologous tumor cell line, expanded in IL-2, and cloned by limiting dilution. One CTL clone, Clone 2, demonstrated HLA-A3 restricted recognition of autologous tumor, but did not recognize an autologous EBV-B cell line or an autologous fibroblast cell line. Limiting dilution appeared to be an important step for the identification of auto-RCC specific CTL activity.

Clone 2 recognized a product of the fibroblast growth factor-5 (FGF-5) gene. Direct sequencing of genomic DNA from autologous EBVB cells showed no mutations in the putative amino acid sequence for FGF-5 in tumor-derived cDNA, indicating that a tumor-specific mutation is not the basis of immune recognition by Clone 2. FGF-5 was not expressed in normal adult organs, and was over-expressed in multiple renal cell carcinomas, prostate carcinomas, and a breast carcinoma. Expression of FGF-5 by renal and other tumors corresponded to the recognition of these tumor cells in an HLA-A3 restricted fashion.

EXAMPLE 1

Isolation of Tumor Infiltrating Lymphocytes (TILs)

This example describes the isolation of TILs from a metastatic RCC lung lesion. Similar methods can be used to isolate TILs from other neoplasms.

The surgically resected remnant of a spontaneously regressing metastatic RCC lung lesion was enzymatically digested (0.1% Collagenase type IV, 30u/ml deoxyribonuclease type IV, and 0.01% hyaluronidase type V [Sigma, St. Louis, MO]) at room temperature (RT) for three hours. After digestion, the cell suspension was filtered through 100 μm nylon mesh and separated by density gradient using Lymphocyte Separation Medium (Organon Teknica, Durham, NC). The lymphocyte-containing interface was recovered, washed with Hanks' Balanced Salt Solution, and used for TIL culture in RPMI (Biofluids, Rockville, MD) 10% human AB serum (Biochemed Pharmacologicals, Winchester, VA) with 6000 IU/ml of recombinant human IL-2 (Chiron Corp.,

Emoryville, CA) in 24 well plates at a cell density of 1 x 10^6 cells/well. The culture was stimulated every two weeks with an irradiated autologous RCC cell line established from a primary left nephrectomy sample (EXAMPLE 2).

An autologous EBV-B cell line was established by EBV infection using a culture supernatant of cell line B95-9 (American Type Culture Collection, ATCC, Manassas, VA). An autologous fibroblast line was established by infecting a one-week old cultured tumor sample with a retrovirus that encoded the papilloma virus E6/E7 proteins and performing limiting dilution. RCC cell lines UOK125, UOK127, UOK130, UOK131, UOK150, and UOK 171 were obtained from Dr. W. Marston Linehan (NCI, Bethesda, MD). Lung cancer cell lines SKGT2, SKGT4, SKGT5 and esophageal cancer cell line HCE-4 were obtained from Dr. David Schrump (NCI, Bethesda, MD). 1570 RCC, 1581 RCC, 1645 RCC, 1764 RCC, CY13, 501 mel, 526 mel, 586 mel, 624.38 mel, 888 mel, 1088 mel, 1199 mel, and 1479 mel were established from surgical samples. SW480, PC-3, and DU 145 were obtained from ATCC (Manassas, VA). TSU-PR1 was obtained from Dr. Suzanne Topalian (NCI, Bethesda, MD).

EXAMPLE 2

Identification of a CTL Clone with Specific Recognition of Autologous RCC

One of the regressing remnants of a metastatic lung lesion of a renal cell carcinoma produced a TIL line when cultured in media with IL-2. This bulk TIL line was stimulated periodically with an irradiated autologous tumor cell line established from a left nephrectomy operation sample. After three months of culture, the autologous tumor-specific T cell clone (Clone 2 CTL) was established by limiting dilution of the bulk TIL line. Clone 2 CTL was $CD3^+$, $CD8^+$ and was found to utilize Vβ12 by PCR-based TCR (T-cell receptor) analysis.

The reactivity of Clone 2 CTL was assessed using an autologous RCC cell line (1764 CTL), an autologous EBV-B cell line, an autologous fibroblast cell line, and other allogeneic RCC cell lines described in EXAMPLE 1. RCC cells (5 x 10^4 cells/well), fibroblasts (5 x 10^4 cells), or EBV-B cells (1 x 10^6 cells/well) were plated into 96 well flat bottom plates and 2 x 10^4 cells/well of Clone 2 CTL were added. After incubating for 20 hours, supernatants were harvested and IFN-γ concentration was analyzed by ELISA (Endogen, Woburn, MA), where IFN-γ concentration is considered proportional to CTL activation.

ELISA plates (96 well flat bottom, Costar, NY) were coated with anti-human IFN-γ monoclonal antibody (2G1, ENDOGEN, MA) at 1 μg/ml, 100 μl/well overnight. After washing, plates were blocked with PBS-5% FBS (fetal bovin serum) for one hour (200 μl/well), the samples added (100 μl/well) and incubated for 90 minutes. Subsequently, the plates were washed and biotin-labeled anti-human IFN-γ monoclonal antibody (B133.5, ENDOGEN, MA) was added at 0.5

μg/ml, 100 μl/well, and incubated for one hour. The plates were subsequently washed and 2000-times diluted HRP-streptavidin conjugate (Zymed Laboratories, CA) was added and incubated for 30 minutes. Plates were washed and DAKO TMB One-Step Substrate System (DAKO Corporation, CA) was added (100 μl/well). The coloration reaction was stopped by adding 0.18 M sulfuric acid (100 μl/well) and the optical density at 450nm wave-length was measured. Recombinant human IFN-γ (ENDOGEN, MA) was used as a standard.

As shown in FIG. 1, the reactivity of Clone 2 CTL with a panel of HLA-typed tumors indicated that Clone 2 CTL recognized an antigen shared among renal cell carcinomas, and this recognition appeared to be restricted by HLA-A3. To confirm the restriction by HLA-A3, a blocking study using HLA-specific monoclonal antibodies (mAbs) was performed. W6/32, MA2.1, and GAPA3 hybridomas were obtained from ATCC and the antibodies purified by Lofstrand Labs (Gaithersburg, MD). B1.23.1 was obtained from NCI, Bethesda, MD. Irradiated tumor cells (5 x 10^4 cells in 100 μl) were incubated with mAbs (20 μg/ml) for 30 minutes at RT, and 2 x 10^4 CTL were added. Following 20 hours of culture at 37°C, the supernatant was assayed for IFN-γ concentration by ELISA as described above. As a control (FIG. 2A), a CTL and autologous tumor target whose interaction is known to be restricted by HLA-A2 was used. The effect of a blocking mAb on the recognition of autologous tumor by Clone 2 CTL is shown in FIG. 2B (anti-class I MHC mAb=W6/32, anti-HLA-A2 mAb=MA2.1, anti-HLA-A3 mAb=GAPA3, anti-HLA-BC mAb=B1.23.1).

As shown in FIG. 2B, tumor recognition by Clone 2 CTL was maximally reduced by a pan-anti-class I MHC mAb (W6/32) and an anti-HLA-A3 mAb (GAPA3). At least 75% inhibition was observed, when compared to the CTL activity of the HLA-A2 restricted CTL. However, blocking by an anti-HLA-A2 mAb (MA2.1) and an anti-HLA-B/C mAb (B1.23.1) was similar to the effect observed with the anti HLA-B/C antibody on an HLA-A2-restricted CTL. Therefore, Clone 2 CTL reactivity is restricted by HLA-A3.

EXAMPLE 3

Cloning the Antigen Recognized by Clone 2 CTL

To identify the gene coding for the antigen recognized by Clone 2 CTL, expression cloning was performed utilizing a plasmid-based cDNA library. Poly $(A)^+$ RNA was prepared from the autologous RCC cell line using a mRNA isolation system (Fast Track kit 2.0; Invitrogen, Carlsbad, CA). cDNA was prepared with the Superscript plasmid system (Life Technologies, Rockville, MD) and ligated into the eukaryotic plasmid expression vector pME18S (Dr. Atsushi Miyajima, University of Tokyo). After electroporation and titration, a cDNA library was prepared in pools by inoculating approximately 100 bacterial clones/well in 1 ml LB/well and purifying

plasmid using the Qiaprep 96 turbo system (Qiagen, Valencia, CA).

To serve as the antigen-presenting target cell line, COS-7 cells were retrovirally transduced with the HLA-A3 gene derived from the autologous tumor cell line. As a control target, the HLA-A0201 gene was retrovirally transduced into COS-7 cells (hereafter referred to as COS-A3 or COS-A2, respectively). To introduce HLA-A3 into non-HLA-A3-expressing cells, the HLA-A3 gene was cloned from autologous 1764 RCC by RT-PCR (forward primer 5'-TTGGGGAGGGAGCACAGGTCAGCGTGGGAAG-3', SEQ ID NO: 24; reverse primer 5'-GGACTCAGAATCTCCCCAGACGCCGAG-3', SEQ ID NO: 25), sequenced, and subcloned into the retroviral vector pRx-IRES-Bsr (Wakimoto *et al.*, *Jpn. J. Cancer Res.* 88:296-305, 1997). Vesicular stomatitis virus G protein-pseudotyped retrovirus was prepared by transiently transfecting the 293 GP cell line using standard methods (see Wang *et al.*, *Cancer Res.* 58:3519-25, 1998, incorporated by reference). Forty-eight hours after transfection, culture supernatant was harvested, filtered, and used for infection with 8 μg/ml of polybrene. Infection efficiencies ranged from 70%-100% and the HLA-A3 positive population was further selected by 5 μg/ml of blasticidin S (Calbiochem, San Diego, CA).

Three independent clones: IA3 (SEQ ID NOS: 7 and 8), 6A4 (SEQ ID NOS: 11 and 12), and 10E4 (SEQ ID NOS: 13-18) selected after subcloning reactive cDNA pools were transfected (100 ng of plasmid) into 5 x 10^4 COS-A2 and COS-A3 using 1 μl of lipofectAMINE (Life Technologies, Rockville, MD) in 96-well plates according to the manufacturer's instructions. The next day, Clone 2 CTL (2 x 10^4 cells/well) were added and after 20 hours incubation, supernatants were assayed for IFN-γ secretion by Clone 2 CTL as described in the above EXAMPLES. An FGF-5 gene from an independent source (FGF-5 MG, from Dr. Mitchell Goldfarb, Mount Sinai School of Medicine) was analyzed the same way.

As shown in FIG 3A, the measured IFN-γ concentration is greater than 2500 pg/ml for the COS-A3 cells, but less than 1 pg/ml for the COS-A2 cells. The clones that conferred recognition of COS-A3 by the CTL (FIG. 3A) were identified and sequenced on an automated sequencer (ABI Prizm 310; Perkin-Elmer Corp., Foster City, CA).

All three clones encoded all or part of an FGF-5 sequence. As shown in FIG. 3B, the sequence of the clones is similar, but not identical to, the human FGF-5 sequence from GenBank Accession No M37825 (MUSFGF5A; SEQ ID NOS 1-4 and FIG. 6). There were eight nucleotide mismatches at positions 79 (A→C), 287 (T→G), 732 (T→G), 810 (T→G), 876 (T→G), 895 (T→C), 974 (A→C) and 975 (A→C), four of which resulted in amino acid changes. The smallest cDNA clone (FIG. 3B) recognized by Clone 2 CTL (1A3-1) (SEQ ID NOS: 7 and 8) had six nucleotide mismatches and two of these changed the amino acid sequence. The longest clone (10E4-1) contained the full-length FGF-5 cDNA sequence (SEQ ID NOS 15 and 17).

The genomic sequence for FGF-5 obtained from autologous EBV-B cells was identical to the autologous tumor-derived sequence for FGF-5. In addition, FGF-5 cDNA from an independent source (FGF-5 MG) was also recognized by Clone 2 CTL when transfected into COS-A3 (FIG. 3A). The DNA sequence of the FGF-5 MG plasmid was identical to clone 1A3-1 (SEQ ID NO: 7). Therefore, the previously published FGF-5 sequence available on GenBank Accession No. M37825 contains at least eight nucleotide and four amino acid differences.

These data demonstrate that Clone 2 CTL recognizes a non-mutated epitope within the 268 amino acid full-length FGF-5. Fragments of FGF-5 were also shown to activate CTL clonal expansion. Fragments of FGF-5 tested are shown in FIG. 7. Fragments which activated CTL clonal expansion are shown in FIG. 7 as open bars, while fragments which did not activate CTL clonal expansion are shown in FIG. 7 as filled bars. Fragment 610-822 did not activate an immune response, while a fragment as short as 60 amino acid residues, 643-822, (SEQ ID NO 19) are immunogenic in HLA-A3 individuals. The region of amino acids 643-679 may be modified in cells. The disclosure therefore provides a number of species of immunogenic petides, and permits one to easily screen for other immunogenic peptides with the assay of this example.

However, other epitopes within the FGF-5 peptide may be immunogenic for individuals who are not HLA-A3$^+$ (for example HLA-A2$^+$ subjects, who constitute about 50% of the Caucasian population). Hence amino acid sequences as short as eight contiguous amino acid residues, for example 8-12, or 20 or less residues of FGF-5 (SEQ ID NOS: 4, 6, 8, 10, 12, and 16-19) are included within the present disclosure. In other embodiments, sequences at least 60 contiguous residues, for example at least 80, 100, 120, 140, 160, 180, 200, 220 or 240 contiguous amino acids of the sequence shown in FIG. 6, or SEQ ID NOS: 3 or 18 are suitable for use with this method.

EXAMPLE 4

Analysis of FGF-5 Expression in Normal and Tumor Cells

To analyze FGF-5 expression, real-time-PCR (RT-PCR) analysis and INF-γ release was measured in normal cells, tumor cells naturally expressing HLA-A3, and in non-HLA-A3 expressing tumors in which HLA-A3 was introduced by retroviral transduction (see EXAMPLE 3). Similar methods can be used to analyze FGF-5 expression in any sample from any organism.

To determine the FGF-5 and β-actin copy number, real-time PCR was used. Total RNA of normal adult human tissues were purchased from Clontech Laboratories (Palo Alto, CA). Total RNA from tumor cell lines were prepared using the RNeasy mini kit according to the manufacturer's instructions (Qiagen, Valencia, CA). First strand cDNA was synthesized using the Superscript Preamplification System (Life Technologies, Rockville, MD) utilizing 5 μg of total

RNA from either normal tissue or tumor cell lines. For the analysis, 2 μl out of 20 μl of the first strand cDNA was used. Two forward primers (FGF-F1 5'-CTTCTTCAGCCACCTGATCCTC (SEQ ID NO: 20) and FGF-F2 5'-TGCAGAGTGGGCATCGGTTTC (SEQ ID NO: 21)) were planned in exon 1 and two reverse primers (FGF-R1 5'-TATGCTCAATGCAGAGGTAC (SEQ ID NO: 22) and FGF-R2 5'-CGTAGTCCCTGTTATTTAAC (SEQ ID NO: 23)) were planned in exon 3.

Using the F1 and R1 pair and Taq polymerase (Life Technologies, Rockville, MD), the first PCR reaction was performed at 94°C for one minute followed by 16 cycles of 94°C for 30 seconds, 61°C for 45 seconds, and 72°C for 60 seconds. As a template for the second nested PCR reaction, 2 μl out of 50 μl of the first PCR reaction was used with the primer pair of F2 and R2 using the same PCR program. The PCR product (10 μl) was subjected to agarose gel analysis. As a control, the expression level of β-actin was also measured.

To measure IFN-γ concentration, tumor cells (5 x 10^4) were plated in flat bottom 96 well plates and 2 x 10^4 Clone 2 CTL were added. After incubating for 20 hours, the supernatants were assayed for IFN-γ concentration by ELISA as described above in EXAMPLE 2.

FGF-5 copy number was normalized to β-actin copy number in each cell line and the FGF-5 copy number/10^5 β-actin copy number plotted (filled bars in FIGS. 4 and 5). In contrast to autologous RCC cell lines (for example 1764 RCC, FIG. 5) which showed strong FGF-5 expression, in the normal tissues analyzed, FGF-5 expression was below the level of detection. As shown in FIG. 4, in normal tissues FGF-5 was only detectable in brain and kidney. However, the FGF-5 copy number in these tissues (about 20 FGF-5 copies/10^5 β-actin copies) was lower than the calculated recognition threshold for CTL clone 2 (FIG. 5). No detectable FGF-5 expression was observed in normal tissues using Northern blotting.

In contrast, as shown in FIG. 5, six of 10 RCC, two of three prostate carcinomas (PC3 and TSU-PR1) and 1 of 2 breast cancers (MDA231) showed significant recognition by CTL clone 2 (as judged by IFN-γ>50 pg/ml, and at least twice that generated against an autologous EBVB control). In addition, these carcinomas showed a higher FGF-5 copy number (bars in FIG. 5) compared with normal tissues (FIG. 4), for example at least 50%, at least 75%, at least 500% or even at least 1000% greater FGF-5 copy number. None of eight malignant melanomas (526, 586, 624.38, 1479, 501, 888, 1088, and 1199 mel), three lung cancers (SKGT-2, 4, and 5), one esophageal carcinoma (HCE-4), and two colon carcinomas (SW480 and CY13) were recognized. As shown in the inset graph of FIG. 5, the recognition by CTL clone 2 highly correlated with FGF-5 copy number ($p<0.0001$). Marginally recognized cells such as 1570 RCC and TSU-PR1 indicates that the FGF-5 expression threshold for recognition by CTL clone 2 was 50-100 FGF-5 mRNA copies/10^5 β actin copies. Therefore, cells having at least 75 FGF-5/10^5 β-actin copies, for

example at least 100 FGF-5/10^5 β-actin copies, for example at least 500 FGF-5/10^5 β-actin copies, for example at least 1000 FGF-5/10^5 β-actin copies, are expected to generate an immune response.

EXAMPLE 5

Immune Therapies

This example describes how the expression of FGF-5 by tumor cells can be used to stimulate the immune system for cancer therapies. In particular embodiments, the immune therapy is preceded by determining whether FGF-5 expression is increased, for example using the method of EXAMPLE 4. The immune therapy can then be administered if FGF-5 expression is found to be increased in the tumor cell. The methods disclosed herein can also be combined with other anti-neoplastic treatments, such as radiotherapy or administration of anti-neoplastic drugs or biologics.

FGF-5 as a Tumor Antigen

FGF-5 satisfies several criteria as a tumor antigen for cancer therapy. It is expressed substantially specifically in tumors but not in normal tissues in humans, particularly adults (FIGS. 4 and 5A and 5B). Although FGF-5 expression has been reported at a low level in brain (Goldfarb *et al. Ann. NY Acad. Sci.* 638:38-52, 1991), FGF-5 was not detected by RT-PCR in brain tissue, even though RT-PCR was sensitive enough to detect FGF-5 expression in tumors. Hence FGF-5 expression in adult human brain is low, if present at all. As an immunologically privileged site, the central nervous system is also unlikely to be an auto-immune target during FGF-5 targeted immunotherapy.

FGF-5 is a non-mutated antigen, over-expressed in some RCC, breast carcinomas, and prostate cancers (FIGS. 5A and 5B). According to the literature, FGF-5 is over-expressed in bladder cancers and pancreatic cancers (Yoshimura *et al.*, *Cancer Lett.* 103:91-7, 1996; Kornman *et al. Oncogene* 15:1417-24, 1997). Therefore it represents an attractive tumor antigen for these cancers, and particularly such cancers which lack identified vaccine targets.

Most tumor-specific antigens are categorized in three groups; 1) tissue differentiation antigens (e.g. MART-1, tyrosinase, gp100); 2) tumor-specific mutations (e.g. β-catenin, CASP-8); and 3) proteins sharing expression on tumor and testis (e.g. MAGE-1, BAGE). Recently antigens overexpressed on tumors but not expressed by testis have been described (e.g. telomerase catalytic subunit (hTERT), p53). FGF-5 is believed to belong to this new class of tumor antigens.

Therapeutics directed against FGF-5

A therapeutic directed against FGF-5 is believed to have substantial therapeutic potential to interfere with important biological functions of tumors, since FGF-5 is believed to have an

important biological function in maintaining the malignant phenotype of a tumor. Examples of such therapies include, but are not limited to immuno-therapeutics such as anti-FGF-5 antibodies (EXAMPLE 9) or the use of antisense molecules which disrupt FGF-5 expression (EXAMPLES 19 AND 20). FGF-5 was originally identified as an oncogene that transformed NIH-3T3 cells (Zhan *et al.*, *Mol. Cell. Biol.* 8:3487-95, 1988). In pancreatic cancer, FGF-5 is believed to have autocrine and paracrine growth-promoting activities, and to function as an angiogenic factor such that it may therefore be suitable as an anti-angiogenic target.

Administration of Autologous CTLs, specific to FGF-5

Therapeutic approaches based upon the disclosure take advantage of a response by a subject's immune system, leading to treatment of cells that express or overexpress FGF-5 (for example lysis or death of the cells, or regression of the tumor), such as HLA-A3 restricted RCC cells. One such therapeutic approach is the administration of autologous CTLs, specific to FGF-5, to a subject with RCC, or other tumor characterized by overexpression of FGF-5. Techniques for obtaining such CTLs *in vitro* are known, as illustrated by the foregoing examples. Autologous peripheral blood mononuclear cells (PBMCs) such as lymphocytes can be used, or cells of dendritic autologous cells, which are stimulated with the FGF-5 antigen.

Generally, a sample of cells taken from a subject, such as blood cells, are contacted with a cell presenting the complex and capable of provoking CTLs to proliferate. In addition to the target cells listed above, the target cell can be a transfectant, such as a COS cell of the type described above. These transfectants present the desired HLA complex on their surface and, when combined with a CTL of interest, stimulate proliferation of the CTL of interest. COS cells, such as those used herein, are widely available, as are other suitable host cells. Specific production of a CTL clone has been described above. The clonally expanded autologous CTLs may then be administered to the subject.

In adoptive transfer, disclosed by Greenberg, *J. Immunol.* 136:1917, 1986; Riddel *et al.*, *Science* 257:238, 1992; Lynch *et al*, *Eur. J. Immunol.* 21:1403-10, 1991; and Kast *et al.*, *Cell* 59:603-14, 1989, cells presenting the desired complex (FGF-5) are combined with CTLs, leading to proliferation of the specific CTLs. The proliferated CTLs are administered to a subject suffering from an FGF-5 over-expressing tumor, such as RCC, carcinoma of the breast, prostate, bladder or pancreas. The CTLs then lyse the FGF-5 expressing tumor cells, and achieve the desired therapeutic goal of assisting in regression of the tumor.

CTLs can also be stimulated *in vivo*, using a number of approaches. One approach is the use of non-proliferative cells expressing the complex of FGF-5 and an HLA presenting molecule (such as HLA-A3), to stimulate an immune response. The cells used in this approach may be those

that normally express the complex, such as irradiated tumor cells or cells transfected with one or both of the genes necessary for presentation of the complex. Chen *et al.*, *Proc. Natl. Acad. Sci. USA* 88:110-4, 1991 exemplifies this approach. Similarly, vectors carrying one or both of the genes of interest may be used, such as viral or bacterial vectors (EXAMPLE 14). For example, nucleic acids which encode FGF-5 may be operably linked to promoter and enhancer sequences which direct expression of the FGF-5 in certain tissues or cell types. The nucleic acid may be incorporated into an expression vector (EXAMPLE 8).

Expression vectors may be unmodified extrachromosomal nucleic acids, plasmids or viral genomes constructed or modified to enable insertion of exogenous nucleic acids, such as those encoding FGF-5. Nucleic acids encoding FGF-5 also may be inserted into a retroviral genome, thereby facilitating integration of the nucleic acid into the genome of the target tissue or cell type. In these systems, the gene of interest is carried by a microorganism, e.g., a Vaccinia virus or retrovirus, which infect host cells. The cells which result present the complex of interest and are recognized by autologous CTLs, which then proliferate.

Administration of FGF-5

A similar effect can be achieved by combining the FGF-5, or a stimulatory fragment thereof, with an adjuvant to facilitate incorporation into cells (such as HLA-A3 presenting cells) *in vivo*. Generally, subjects receive an intradermal injection of an effective amount of the FGF-5, and/or immunogenic fragments or variants derived therefrom. Initial doses can be followed by booster doses, following immunization protocols standard in the art.

As part of the immunization protocols, substances which potentiate the immune response may be administered with nucleic acid or peptide components of a cancer vaccine. Such immune response potentiating compounds may be classified as either adjuvants or cytokines. Adjuvants may enhance the immunological response by providing a reservoir of antigen (extracellularly or within macrophages), activating macrophages and stimulating specific sets of lymphocytes. Adjuvants of many kinds are well known in the art; specific examples include MPL (SmithKline Beecham), a congener obtained after purification and acid hydrolysis of Salmonella minnesota Re 595 lipopolysaccharide, QS21 (SmithKline Beecham), a pure QA-21 saponin purified from Quillja saponaria extract, and various water-in-oil emulsions prepared from biodegradable oils such as squalene and/or tocopherol. Cytokines are also useful in vaccination protocols as a result of lymphocyte stimulatory properties. Many cytokines useful for such purposes will be known to one of ordinary skill in the art, including interleukin-12 (IL-12) which has been shown to enhance the protective effects of vaccines (*Science* 268: 1432-4, 1995).

When administered, the therapeutic compositions of the present disclosure may be, for example, administered in pharmaceutically acceptable preparations (EXAMPLE 18). Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents (including other anti-neoplastic agents).

When it is desired to stimulate a cytotoxic lymphocyte immune response using a therapeutic composition disclosed herein, it is believed that doses of immunogens ranging from one nanogram/kilogram to 100 miligrams/kilogram, depending upon the mode of administration, would be effective. The range may, for example, be between 500 nanograms and 500 micrograms per kilogram. The absolute amount will depend upon a variety of factors, including the material selected for administration, whether the administration is in single or multiple doses, and individual subject parameters including age, physical condition, size, weight, and the stage of the disease. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation.

EXAMPLE 6

Probes, Primers and DNA Encoding FGF-5

Nucleic acid probes and primers can be readily prepared based on the nucleic acid molecules provided herein (SEQ ID NOS: 3, 5, 7, 9, 11, 13-15, 17 and FIG. 6). A probe comprises an isolated nucleic acid attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, enzyme substrates, co-factors, ligands, chemiluminescent or fluorescent agents, haptens, and enzymes. Methods for labeling and guidance in the choice of labels appropriate for various purposes are discussed, *e.g.*, in Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989) and Ausubel *et al.* (In *Current Protocols in Molecular Biology*, Greene Publ. Assoc. and Wiley-Intersciences, 1992).

Primers are short nucleic acid molecules, such as DNA oligonucleotides 15 nucleotides or more in length. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then the primer extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, *e.g.*, by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art.

Methods for preparing and using probes and primers are described, for example, in Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989), Ausubel *et al.* (In *Current Protocols in Molecular Biology*, Greene Publ. Assoc. and Wiley-

Intersciences, 1992), and Innis *et al.* (*PCR Protocols, A Guide to Methods and Applications*, Academic Press, Inc., San Diego, CA, 1990). PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, MA). One of ordinary skill in the art will appreciate that the specificity of a particular probe or primer increases with its length. Thus, for example, a primer comprising 20 consecutive nucleotides of the *FGF-5* gene will anneal to a target sequence. Thus, in order to obtain greater specificity, probes and primers can be selected that comprise 20, 25, 30, 35, 40, 50 or more consecutive nucleotides of FGF-5 gene sequences.

The disclosure thus includes isolated nucleic acid molecules that comprise specified lengths of the disclosed gene sequences. Such molecules may comprise at least 20, 25, 30, 35, 40 or 50 consecutive nucleotides of these sequences, and may be obtained from any region of the disclosed sequences. By way of example, the gene sequences may be apportioned into halves or quarters based on sequence length, and the isolated nucleic acid molecules may be derived from the first or second halves of the molecules, or any of the four quarters.

The probes and primers can be used for the detection of FGF-5 DNA and the detection of specific mutations or polymorphisms associated with tumor development or prognosis.

EXAMPLE 7

FGF-5 Sequence Variants

The amino acid sequences of FGF-5 (SEQ ID NOS: 4, 6, 8, 10, 12, and 16-19 and FIG. 6) encoded by FGF-5 cDNAs (SEQ ID NOS: 3, 5, 7, 9, 11, 13-15, 17 and FIGS. 3C and 6) are disclosed. The distinctive functional characteristic of FGF-5 is its ability to modulate an immune response, for example the ability to stimulate an HLA-A3-restricted CTL immune response against FGF-5 expressing or over-expressing tumors, as measured by IFN-γ concentration. This activity is reduced by about at least 50%, for example 75%, in the presence of pan-anti-class I MHC mAb (such as W6/32) or anti-HLA-A3 mAb (such as GAPA3). Another distinctive functional characteristic of FGF-5 is the observation that FGF-5 is expressed at undetectable levels in normal adult tissues, but is expressed or over-expressed in some cancers, such as RCC, prostate and breast carcinomas. These activities of FGF-5 proteins may readily be determined using the assays described above, for example those described in EXAMPLES 2-4.

Having presented the nucleotide sequence of FGF-5 cDNAs and the amino acid sequence of these proteins, this disclosure facilitates the creation of DNA molecules, and thereby proteins, which are derived from those disclosed but which vary in their precise nucleotide or amino acid

sequence from those disclosed. Such variants may be obtained through a combination of standard molecular biology laboratory techniques and the nucleotide sequence information disclosed herein.

FGF-5 variants, polymorphisms, mutants, and fragments will retain the ability to modulate an immune response, for example stimulate an immune response, for example, an HLA-A3-restricted CTL immune response. Such an immune response in particular embodiments, induces a regression of the FGF-5 expressing tumor. In other embodiments, in the presence of pan-anti-class I MHC mAb (such as W6/32) or anti-HLA-A3 mAb (such as GAPA3) and FGF-5 variants, polymorphisms, mutants, and fragments, the stimulated immune response, such as an HLA-A3-restricted CTL immune response, is blocked. This as observed by an at least 50% decrease in IFN-γ concentration using the assay described in EXAMPLE 2.

Substitutions of FGF-5 amino acid sequences can be made either in regions that are highly conserved between species, or regions that share less conservation between species. In particular embodiments regions of FGF-5 which are highly conserved between species ideally do not substantially diverge from the wild-type sequence shown in SEQ ID NOS: 4, 6, 8, 10, 12, and 16-19 and FIG. 6. Other important residues include the receptor binding domain and the heparin binding domain (both located in the center of the protein). Other important residues include amino acids 161-220 of FGF-5 (shown in SEQ ID NO: 19). In these regions, conservative substitutions will be better tolerated than non-conservative substitutions. Alterations in regions that are less conserved are predicted to have less of an effect on the function of the FGF-5 protein. Eight examples of differences among the FGF-5 sequences are at positions 79 (A→C), 287 (T→G), 732 (T→G), 810 (T→G), 876 (T→G), 895 (T→C), 974 (A→C) and 975 (A→C). Thus, the methods disclosed herein may be practiced with molecules that differ from the exact molecules disclosed, but which retain the requisite ability to modulate an immune response or FGF-5 expression or activity.

Variants and fragments may retain at least 60%, 70%, 75% 80%, 85%, 90%, 95%, 98%, or greater sequence identity to the FGF-5 amino acid sequences disclosed herein, and in particular embodiments at least this much identity to SEQ ID NOS: 4, 6, 8, 10, 12, and 16-19 and FIG. 6. Less identity is allowed, as long as the variant FGF-5 sequence maintains the functional activity of the FGF-5 protein as defined herein. Such activity can be readily determined using the assays disclosed herein.

The simplest modifications involve the substitution of one or more amino acid residues (for example 2, 5 or 10 residues) for amino acid residues having similar biochemical properties. These so-called conservative substitutions are likely to have minimal impact on the activity of the resultant protein. Substitutional variants are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions

generally are conservative when it is desired to finely modulate the characteristics of the protein. Examples of amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative substitutions include: Ser for Ala; Lys for Arg; Gln or His for Asn; Glu for Asp; Ser for Cys; Asn for Gln; Asp for Glu; Pro for Gly; Asn or Gln for His; Leu or Val for Ile; Ile or Val for Leu; Arg or Gln for Lys; Leu or Ile for Met; Met, Leu or Tyr for Phe; Thr for Ser; Ser for Thr; Tyr for Trp; Trp or Phe for Tyr; and Ile or Leu for Val.

Amino acid substitutions are typically of single residues, for example 1, 2, 3, 4, 5, 10 or more substitutions; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. Obviously, the mutations that are made in the DNA encoding the protein must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure.

Changes in function or immunological identity (increase or decrease) are made by selecting substitutions that are less conservative than those listed above, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine. Such FGF-5 variants can be readily selected for additional testing by performing an assay (such as that shown in EXAMPLES 2-4) to determine if the FGF-5 variant retains the ability to stimulate an HLA-A3-restricted CTL immune response against FGF-5 expressing or over-expressing tumors, as measured by IFN-γ concentration.

Variant DNA molecules include those created by standard DNA mutagenesis techniques, for example, M13 primer mutagenesis. Details of these techniques are provided in Sambrook *et al.* (In: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989, Chapter 15, herein incorporated by reference). By the use of such techniques, variants may be created which differ in minor ways from those disclosed. DNA molecules and nucleotide sequences which are derivatives of those specifically disclosed herein and which differ from those disclosed by the

deletion, addition or substitution of nucleotides while still encoding a protein which possesses the functional characteristics of FGF-5 proteins are comprehended by this disclosure.

Also disclosed are small DNA molecules which are derived from the disclosed DNA molecules. Such small DNA molecules include oligonucleotides suitable for use as hybridization probes or PCR primers. As such, these small DNA molecules will comprise at least a segment of FGF-5 cDNA molecules or FGF-5 genes. For example, the sequences will comprise at least 20, 25, 30, 40, or 50 contiguous nucleotides of nucleotide sequence of FGF-5 (SEQ ID NOS: 3, 5, 7, 9, 11, 13-15, 17 and FIG. 6, or their complementary strands) (i.e., at least 20-50 consecutive nucleotides of the FGF-5 cDNA/gene sequences). It will be appreciated that such longer length nucleotide sequences will provide greater specificity in hybridization or PCR applications than shorter length sequences. Accordingly, superior results may be obtained using these longer stretches of consecutive nucleotides.

DNA molecules and nucleotide sequences which are derived from the disclosed DNA molecules as described above may also be defined as DNA sequences which hybridize under stringent conditions to the DNA sequences disclosed, or fragments thereof. Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the hybridization method of choice and the composition and length of the hybridizing DNA used. Generally, the temperature of hybridization and the ionic strength (especially the Na^+ concentration) of the hybridization buffer will determine the stringency of hybridization. Calculations regarding hybridization conditions required for attaining particular degrees of stringency are discussed by Sambrook *et al.* (In: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989 ch. 9 and 11), herein incorporated by reference.

Specific hybridization refers to the binding, duplexing, or hybridizing of a molecule only or substantially only to a particular nucleotide sequence when that sequence is present in a complex mixture (e.g. total cellular DNA or RNA). Specific hybridization may also occur under conditions of varying stringency.

Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the hybridization method of choice and the composition and length of the hybridizing DNA used. Generally, the temperature of hybridization and the ionic strength (especially the Na^+ concentration) of the hybridization buffer will determine the stringency of hybridization. Calculations regarding hybridization conditions required for attaining particular degrees of stringency are discussed by Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989 chapters 9 and 11).

By way of illustration only, a hybridization experiment may be performed by hybridization of a DNA molecule (for example, a variant of a FGF-5 cDNA) to a target DNA molecule (for

example, a FGF-5 cDNA) which has been electrophoresed in an agarose gel and transferred to a nitrocellulose membrane by Southern blotting (Southern, *J. Mol. Biol.* 98:503, 1975), a technique well known in the art and described in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor, New York, 1989).

Hybridization with a target probe labeled, for example, with [^{32}P]-dCTP is generally carried out in a solution of high ionic strength such as 6xSSC at a temperature that is 20-25°C below the melting temperature, T_m, described below. For such Southern hybridization experiments where the target DNA molecule on the Southern blot contains 10 ng of DNA or more, hybridization is typically carried out for 6-8 hours using 1-2 ng/ml radiolabeled probe (of specific activity equal to 10^9 CPM/μg or greater). Following hybridization, the nitrocellulose filter is washed to remove background hybridization. The washing conditions should be as stringent as possible to remove background hybridization but to retain a specific hybridization signal.

The term T_m represents the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Because the target sequences are generally present in excess, at T_m, 50% of the probes are occupied at equilibrium. The T_m of such a hybrid molecule may be estimated from the following equation (Bolton and McCarthy, *Proc. Natl. Acad. Sci. USA* 48:1390, 1962): $T_m = 81.5°C - 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - 0.63(\%\text{ formamide}) - (600/l)$; where l = the length of the hybrid in base pairs.

This equation is valid for concentrations of Na^+ in the range of 0.01 M to 0.4 M, and it is less accurate for calculations of T_m in solutions of higher $[Na^+]$. The equation is also primarily valid for DNAs whose G+C content is in the range of 30% to 75%, and it applies to hybrids greater than 100 nucleotides in length (the behavior of oligonucleotide probes is described in detail in Ch. 11 of Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989).

Thus, by way of example, for a 150 base pair DNA probe with a hypothetical GC content of 45%, a calculation of hybridization conditions required to give particular stringencies may be made as follows. For this example, it is assumed that the filter will be washed in 0.3 xSSC solution following hybridization, thereby: $[Na^+] = 0.045$ M; %GC = 45%; Formamide concentration = 0; l = 150 base pairs; $T_m = 81.5 - 16.6(\log_{10}[Na^+]) + (0.41 \times 45) - (600/150)$; and so $T_m = 74.4°C$.

The T_m of double-stranded DNA decreases by 1-1.5°C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol.* 81:123, 1973). Therefore, for this given example, washing the filter in 0.3 xSSC at 59.4-64.4°C will produce a stringency of hybridization equivalent to 90%; that is, DNA molecules with more than 10% sequence variation relative to the target

cDNA will not hybridize. Alternatively, washing the hybridized filter in 0.3 xSSC at a temperature of 65.4-68.4°C will yield a hybridization stringency of 94%; that is, DNA molecules with more than 6% sequence variation relative to the target cDNA molecule will not hybridize. The above example is given entirely by way of theoretical illustration. One skilled in the art will appreciate that other hybridization techniques may be utilized and that variations in experimental conditions will necessitate alternative calculations for stringency.

Examples of stringent conditions are those under which DNA molecules with more than 25%, 15%, 10%, 6% or 2% sequence variation (also termed "mismatch") will not hybridize. Stringent conditions are sequence dependent and are different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An example of stringent conditions is no more than about 5°C lower than the thermal melting point T_m for the specific sequence at a defined ionic strength and pH. An example of stringent conditions is a salt concentration of at least about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and a temperature of at least about 30°C for short probes (e.g. 10 to 50 nucleotides). Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. For example, conditions of 5X SSPE (750 mM NaCl, 50 mM Na Phosphate, 5 mM EDTA, pH 7.4) and a temperature of 25-30°C are suitable for allele-specific probe hybridizations.

A perfectly matched probe has a sequence perfectly complementary to a particular target sequence. The test probe is typically perfectly complementary to a portion (subsequence) of the target sequence. The term "mismatch probe" refers to probes whose sequence is deliberately selected not to be perfectly complementary to a particular target sequence.

Transcription levels can be quantitated absolutely or relatively. Absolute quantitation can be accomplished by inclusion of known concentrations of one or more target nucleic acids (for example control nucleic acids such as Bio B or with a known amount the target nucleic acids themselves) and referencing the hybridization intensity of unknowns with the known target nucleic acids (for example by generation of a standard curve).

The degeneracy of the genetic code further widens the scope of the disclosure as it enables major variations in the nucleotide sequence of a DNA molecule while maintaining the amino acid sequence of the encoded protein. For example, the eighteenth amino acid residue of FGF-5 protein is alanine. This is encoded in the FGF-5 cDNA by the nucleotide codon triplet GCC. Because of the degeneracy of the genetic code, three other nucleotide codon triplets, GCT, GCA and GCG, also code for alanine. Thus, the nucleotide sequence of the FGF-5 cDNA could be changed at this position to any of these three codons without affecting the amino acid composition of the encoded protein or the characteristics of the protein. Based upon the degeneracy of the genetic code, variant DNA molecules may be derived from the cDNA molecules disclosed herein using standard DNA

mutagenesis techniques as described above, or by synthesis of DNA sequences. DNA sequences which do not hybridize under stringent conditions to the cDNA sequences disclosed by virtue of sequence variation based on the degeneracy of the genetic code are herein also comprehended by this disclosure.

One skilled in the art will recognize that the DNA mutagenesis techniques described above may be used not only to produce variant DNA molecules, but will also facilitate the production of proteins which differ in certain structural aspects from FGF-5 proteins, yet which proteins are clearly derivative of this protein and which maintain the essential characteristics of a FGF-5 protein as defined above. Newly derived proteins may also be selected in order to obtain variations on the characteristic of a FGF-5 protein, as will be more fully described below. Such derivatives include those with variations in amino acid sequence including minor deletions, additions and substitutions.

While the site for introducing an amino acid sequence variation is predetermined, the mutation per se need not be predetermined. For example, in order to optimize the performance of a mutation at a given site, random mutagenesis may be conducted at the target codon or region and the expressed protein variants screened for optimal activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence as described above are well known.

FGF-5 genes, cDNA, DNA molecules derived therefrom and the protein encoded by these cDNAs and derivative DNA molecules may be utilized in aspects of both the study of FGF-5 and for diagnostic and therapeutic applications related to FGF-5. Those skilled in the art will recognize that the utilities herein described are not limited to the specific experimental modes and materials presented and will appreciate the wider potential utility of this disclosure.

EXAMPLE 8

Recombinant Expression of FGF-5

With the provision of FGF-5 cDNAs (SEQ ID NOS: 3, 5, 7, 9, 11, 13-15, 17 and FIGS. 3C and 6) and amino acid sequences (SEQ ID NOS: 4, 6, 8, 10, 12, and 16-19 and FIG. 6), the expression and purification of the corresponding proteins by standard laboratory techniques is now enabled. The purified protein may be used for functional analyses, antibody production and therapy in a subject as described herein. Furthermore, the DNA sequence of FGF-5 cDNAs and any variant or fragment thereof, can be manipulated in studies to understand the expression of the gene and the function of its product.

Partial or full-length cDNA sequences encoding an FGF-5 protein, may be ligated into bacterial expression vectors. Methods for expressing large amounts of protein from a cloned gene introduced into *E. coli* may be utilized for the purification, localization and functional analysis of

proteins. For example, fusion proteins consisting of amino terminal peptides encoded by a portion of the *E. coli lacZ* or *trpE* gene linked to FGF-5 may be used to prepare polyclonal and monoclonal antibodies against FGF-5. Thereafter, these antibodies may be used to purify proteins by immunoaffinity chromatography, in diagnostic assays to quantitate the levels of protein, to localize FGF-5 in tissues and individual cells by immunofluorescence and as a therapeutic to treat FGF-5 expressing or over-expressing tumors.

Intact native protein, or variants or fragments thereof, may also be produced in *E. coli* in large amounts for functional studies. Methods and plasmid vectors for producing fusion proteins and intact native proteins in bacteria are described in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989, Chapter 17, herein incorporated by reference). Such fusion proteins may be made in large amounts, are easy to purify, and can be used to elicit antibody response. Native proteins can be produced in bacteria by placing a strong, regulated promoter and an efficient ribosome binding site upstream of the cloned gene. If low levels of protein are produced, additional steps may be taken to increase protein production; if high levels of protein are produced, purification is relatively easy. Suitable methods are presented in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989) and are well known in the art. Often, proteins expressed at high levels are found in insoluble inclusion bodies. Methods for extracting proteins from these aggregates are described by Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989, Chapter 17).

Vector systems suitable for the expression of lacZ fusion genes include the pUR series of vectors (Ruther and Muller-Hill, 1983, *EMBO J.* 2:1791), pEX1-3 (Stanley and Luzio, 1984, *EMBO J.* 3:1429) and pMR100 (Gray *et al.*, 1982, *Proc. Natl. Acad. Sci. USA* 79:6598). Vectors suitable for the production of intact native proteins include pKC30 (Shimatake and Rosenberg, 1981, *Nature* 292:128), pKK177-3 (Amann and Brosius, 1985, *Gene* 40:183) and pET-3 (Studiar and Moffatt, 1986, *J. Mol. Biol.* 189:113). FGF-5 fusion proteins may be isolated from protein gels, lyophilized, ground into a powder and used as an antigen. The DNA sequence can also be transferred to other cloning vehicles, such as other plasmids, bacteriophages, cosmids, animal viruses and yeast artificial chromosomes (YACs) (Burke et al., 1987, *Science* 236:806-12). These vectors may then be introduced into a variety of hosts including somatic cells, and simple or complex organisms, such as bacteria, fungi (Timberlake and Marshall, 1989, *Science* 244:1313-7), invertebrates, plants (Gasser and Fraley, 1989, Science 244:1293), and mammals (Pursel *et al.*, 1989, *Science* 244:1281-8), which cell or organisms are rendered transgenic by the introduction of the heterologousFGF-5 cDNA.

For expression in mammalian cells, the cDNA sequence may be ligated to heterologous promoters, such as the simian virus SV40, promoter in the pSV2 vector (Mulligan and Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072-6), and introduced into cells, such as monkey COS-1 cells (Gluzman, 1981, *Cell* 23:175-82), to achieve transient or long-term expression. The stable integration of the chimeric gene construct may be maintained in mammalian cells by biochemical selection, such as neomycin (Southern and Berg, 1982, *J. Mol. Appl. Genet.* 1:327-41) and mycophoenolic acid (Mulligan and Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072-6).

DNA sequences can be manipulated with standard procedures such as restriction enzyme digestion, fill-in with DNA polymerase, deletion by exonuclease, extension by terminal deoxynucleotide transferase, ligation of synthetic or cloned DNA sequences, site-directed sequence-alteration via single-stranded bacteriophage intermediate or with the use of specific oligonucleotides in combination with PCR.

The cDNA sequence (or portions derived from it) or a mini gene (a cDNA with an intron and its own promoter) may be introduced into eukaryotic expression vectors by conventional techniques. These vectors are designed to permit the transcription of the cDNA eukaryotic cells by providing regulatory sequences that initiate and enhance the transcription of the cDNA and ensure its proper splicing and polyadenylation. Vectors containing the promoter and enhancer regions of the SV40 or long terminal repeat (LTR) of the Rous Sarcoma virus and polyadenylation and splicing signal from SV40 are readily available (Mulligan and Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072-6; Gorman *et al.*, 1982, *Proc. Natl. Acad. Sci USA* 78:6777-81). The level of expression of the cDNA can be manipulated with this type of vector, either by using promoters that have different activities (for example, the baculovirus pAC373 can express cDNAs at high levels in *S. frugiperda* cells (Summers and Smith, 1985, *Genetically Altered Viruses and the Environment*, Fields *et al.* (Eds.) 22:319-328, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.) or by using vectors that contain promoters amenable to modulation, for example, the glucocorticoid-responsive promoter from the mouse mammary tumor virus (Lee *et al.*, 1982, Nature 294:228). The expression of the cDNA can be monitored in the recipient cells 24 to 72 hours after introduction (transient expression).

In addition, some vectors contain selectable markers such as the *gpt* (Mulligan and Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072-6) or *neo* (Southern and Berg, 1982, *J. Mol. Appl. Genet.* 1:327-41) bacterial genes. These selectable markers permit selection of transfected cells that exhibit stable, long-term expression of the vectors (and therefore the cDNA). The vectors can be maintained in the cells as episomal, freely replicating entities by using regulatory elements of viruses such as papilloma (Sarver et al., 1981, *Mol. Cell Biol.* 1:486) or Epstein-Barr (Sugden *et al.*, 1985, *Mol. Cell Biol.* 5:410). Alternatively, one can also produce cell lines that have

integrated the vector into genomic DNA. Both of these types of cell lines produce the gene product on a continuous basis. One can also produce cell lines that have amplified the number of copies of the vector (and therefore of the cDNA as well) to create cell lines that can produce high levels of the gene product (Alt *et al.*, 1978, *J. Biol. Chem.* 253:1357).

The transfer of DNA into eukaryotic, in particular human or other mammalian cells, is now a conventional technique. The vectors are introduced into the recipient cells as pure DNA (transfection) by, for example, precipitation with calcium phosphate (Graham and vander Eb, 1973, *Virology* 52:466) or strontium phosphate (Brash et al., 1987, *Mol. Cell Biol.* 7:2013), electroporation (Neumann *et al.*, 1982, *EMBO J.* 1:841), lipofection (Felgner *et al.*, 1987, *Proc. Natl. Acad. Sci USA* 84:7413), DEAE dextran (McCuthan *et al.*, 1968, *J. Natl. Cancer Inst.* 41:351), microinjection (Mueller *et al.*, 1978, *Cell* 15:579), protoplast fusion (Schafner, 1980, *Proc. Natl. Acad. Sci. USA* 77:2163-7), or pellet guns (Klein *et al.*, 1987, *Nature* 327:70). Alternatively, the cDNA can be introduced by infection with virus vectors. Systems are developed that use, for example, retroviruses (Bernstein *et al.*, 1985, *Gen. Engrg.* 7:235), adenoviruses (Ahmad *et al.*, 1986, *J. Virol.* 57:267), or Herpes virus (Spaete *et al.*, 1982, *Cell* 30:295).

These eukaryotic expression systems can be used for studies of FGF-5 genes and mutant forms of these genes, FGF-5 proteins and mutant forms of these proteins. Such uses include, for example, the identification of regulatory elements located in the 5' region of FGF-5 genes on genomic clones that can be isolated from genomic DNA libraries, such as human or other non-human primate libraries, using the information contained in the present disclosure. The eukaryotic expression systems may also be used to study the function of the normal complete protein, specific portions of the protein, or of naturally occurring or artificially produced mutant proteins. Naturally occurring mutant proteins may exist in a variety of diseases in which apoptosis has become disregulated, while artificially produced mutant proteins can be designed by site directed mutagenesis as described above. These latter studies may probe the function of any desired amino acid residue in the protein by mutating the nucleotide coding for that amino acid.

Using the above techniques, the expression vectors containing FGF-5 genes or cDNA sequences or variants, fragments, or mutants thereof, can be introduced into human cells, mammalian cells from other species or non-mammalian cells as desired. The choice of cell is determined by the purpose of the treatment. For example, monkey COS cells (Gluzman, 1981, *Cell* 23:175-82) that produce high levels of the SV40 T antigen and permit the replication of vectors containing the SV40 origin of replication may be used. Similarly, Chinese hamster ovary (CHO), mouse NIH 3T3 fibroblasts or human fibroblasts or lymphoblasts may be used.

One method that can be used to express FGF-5 polypeptides from cloned FGF-5 cDNA sequences in mammalian cells is to use the cloning vector, pXTI (Stratagene,), which contains the

Long Terminal Repeats (LTRs) and a portion of the GAG gene from Moloney Murine Leukemia Virus. The position of the viral LTRs allows highly efficient, stable transfection of the region within the LTRs. The vector also contains the Herpes Simplex Thymidine Kinase promoter (TK), active in embryonal cells and in a wide-variety of tissues in mice, and a selectable neomycin gene conferring G418 resistance. Two unique restriction sites BglII and XhoI are directly downstream from the TK promoter. FGF-5 cDNA, including the entire open reading frame for the FGF-5 proteins and the 3' untranslated region of the cDNAs are cloned into one of the two unique restriction sites downstream from the promoter.

The ligated product is transfected into mouse NIH 3T3 cells using Lipofectin (Life Technologies, Inc.) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in 600 μg/ml G418 (Sigma, St. Louis, MO). The protein is released into the supernatant and may be purified by standard immunoaffinity chromatography techniques using antibodies raised against FGF-5 proteins (see EXAMPLE 9).

Expression of FGF-5 proteins, variants, polymorphisms, fragments of variants thereof, in eukaryotic cells can be used as a source of proteins to raise antibodies. The proteins may be extracted following release of the protein into the supernatant as described above, or, the cDNA sequence may be incorporated into a eukaryotic expression vector and expressed as a chimeric protein with, for example, β-globin. Antibody to β-globin is thereafter used to purify the chimeric protein. Corresponding protease cleavage sites engineered between the β-globin gene and the cDNA are then used to separate the two polypeptide fragments from one another after translation. One useful expression vector for generating β-globin chimeric proteins is pSG5 (Stratagene). This vector encodes rabbit β-globin.

The recombinant cloning vector then comprises the selected DNA of the DNA sequences disclosed herein for expression in a suitable host. The DNA is operatively linked in the vector to an expression control sequence in the recombinant DNA molecule so that the FGF-5 polypeptide can be expressed. The expression control sequence may be selected from the group consisting of sequences that control the expression of genes of prokaryotic or eukaryotic cells and their viruses and combinations thereof. The expression control sequence may be specifically selected from the group consisting of the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the early and late promoters of SV40, promoters derived from polyoma, adenovirus, retrovirus, baculovirus and simian virus, the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, the promoter of the yeast alpha-mating factors and combinations thereof.

The host cell, which may be transfected with the vector disclosed herein, may be selected from the group consisting of bacteria, yeast, fungi, plant, insect, mouse or other animal subject; or human tissue cells.

It is appreciated that for mutant or variant DNA sequences, similar systems are employed to express and produce the mutant or variant product.

EXAMPLE 9

Production and Use of Antibodies

This example describes several methods than can be used to produce antibodies that recognize FGF-5. Such antibodies can be used to purify proteins by immunoaffinity chromatography, in diagnostic assays to quantitate FGF-5 levels, to localize FGF-5 in tissues and individual cells for example by immunofluorescence, and as a therapeutic agent to treat FGF-5 expressing or over-expressing tumors.

Production of Antibodies

Monoclonal or polyclonal antibodies may be produced to either normal FGF-5 proteins, or variants, polymorphisms, fragments and mutant forms thereof. Optimally, antibodies raised against the protein will specifically detect the protein. That is, antibodies raised against the protein (e.g. FGF-5) would recognize and bind the protein and would not substantially recognize or bind to other cellular proteins (such as serum albumin). The determination that an antibody specifically detects an FGF-5 protein is made by any one of a number of standard immunoassay methods; for instance, the Western blotting technique (Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

To determine that a given antibody preparation (such as one produced in a mouse against the FGF-5 protein) specifically detects the FGF-5 protein by Western blotting, total cellular protein is extracted from cells (for example, lymphocytes) and electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel. The proteins are then transferred to a membrane (for example, nitrocellulose) by Western blotting, and the antibody preparation is incubated with the membrane. After washing the membrane to remove non-specifically bound antibodies, the presence of specifically bound antibodies is detected by the use of an anti-mouse antibody conjugated to an enzyme such as alkaline phosphatase; application of the substrate 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium results in the production of a dense blue compound by immuno-localized alkaline phosphatase.

Antibodies which specifically detect FGF-5 proteins will, by this technique, be shown to bind to the protein band, which will be localized at a given position on the gel determined by its

molecular weight. Non-specific binding of the antibody to other proteins may occur and may be detectable as a weak signal on the Western blot. The non-specific nature of this binding will be recognized by one skilled in the art by the weak signal obtained on the Western blot relative to the strong primary signal arising from the specific antibody-FGF-5 protein binding.

Antibodies that specifically bind to an FGF-5 protein (or any of the other novel proteins disclosed herein) belong to a class of molecules that are referred to herein as "specific binding agents." Specific binding agents that are capable of specifically binding to a FGF-5 protein may include polyclonal antibodies, monoclonal antibodies (including humanized monoclonal antibodies) and fragments of monoclonal antibodies such as Fab, F(ab')2 and Fv fragments, as well as any other agent capable of specifically binding to a FGF-5 protein (or the other disclosed proteins).

Substantially pure FGF-5 protein suitable for use as an immunogen is isolated from the transfected or transformed cells as described. Concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms per milliliter. Monoclonal or polyclonal antibody to the protein can then be prepared as follows.

Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of FGF-5 proteins, identified and isolated as described, can be prepared from murine hybridomas according to the classical method of Kohler and Milstein (*Nature* 256:495, 1975) or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein (or epitope thereof) over a period of a few weeks. The mouse is then sacrificed, and the antibody-producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall (*Enzymol.* 70:419, 1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Harlow and Lane (*Antibodies: A Laboratory Manual*. 1988, Cold Spring Harbor Laboratory, New York). In addition, protocols for producing humanized forms of monoclonal antibodies (for therapeutic applications) and fragments of monoclonal antibodies are known in the art.

Polyclonal Antibody Production by Immunization

Polyclonal antiserum containing antibodies to heterogeneous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than others and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis *et al.* (*J. Clin. Endocrinol. Metab.* 33:988-91, 1971).

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony *et al.* (In: *Handbook of Experimental Immunology*, Wier, D. (ed.). Chapter 19. Blackwell. 1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 μM). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher (*Manual of Clinical Immunology*, Chapter 42. 1980).

Synthetic Peptides

Another approach to raising antibodies against FGF-5 proteins is to use synthetic peptides synthesized on a commercially available peptide synthesizer based upon the predicted amino acid sequence of FGF-5 proteins.

Antibodies may be raised against FGF-5 proteins by subcutaneous injection of a DNA vector which expresses a FGF-5 protein into laboratory animals, such as mice. Delivery of the recombinant vector into the animals may be achieved using a hand-held form of the Biolistic system (Sanford *et al.*, 1987, *Pariculate Sci. Technol.* 5:27-37) as described by Tang *et al.* (*Nature*, 356:152-4, 1992). Expression vectors suitable for this purpose may include those which express a FGF-5 cDNA under the transcriptional control of either the human actin promoter or the cytomegalovirus (CMV) promoter.

Antibody preparations prepared according to these protocols are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples;

they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample.

Antibodies Raised by Injection of FGF-5 cDNA

Antibodies may be raised against FGF-5 proteins by subcutaneous injection of a DNA vector which expresses FGF-5 protein (or variant, fragment, polymorphism, or mutant thereof) into laboratory animals, such as mice. Delivery of the recombinant vector into the animals may be achieved using a hand-held form of the Biolistic system (Sanford *et al.*, *Particulate Sci. Technol.* 5:27-37, 1987) as described by Tang *et al.* (*Nature* 356:152-4, 1992). Expression vectors suitable for this purpose may include those which express the FGF-5 cDNA under the transcriptional control of either the human β-actin promoter or the CMV promoter.

Antibody preparations prepared according to these protocols are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample.

Labeled Antibodies

Antibodies disclosed herein can be conjugated with various labels for their direct detection (see Chapter 9, Harlow and Lane, *Antibodies: A Laboratory Manual*. 1988). The label, which may include, but is not limited to, a radiolabel, enzyme, fluorescent probe, or biotin, is chosen based on the method of detection available to the user.

Antibodies can be radiolabeled with iodine (^{125}I), which yields low-energy gamma and X-ray radiation. Briefly, 10 μg of protein in 25 μl of 0.5 M sodium phosphate (pH 7.50 is placed in a 1.5 ml conical tube. To this, 500 μC of $Na^{125}I$, and 25 μl of 2 mg/ml chloramine T is added and incubated for 60 seconds at RT. To stop the reaction, 50μl of chloramine T stop buffer is added (2.4 mg/ml sodium metabisulfite, 10 mg/ml tyrosine, 10% glycerol, 0.1% xylene cyanol in PBS). The iodinated antibody is separated from the iodotyrosine on a gel filtration column. Antibodies disclosed herein can also be labeled with biotin, with enzymes such as alkaline phosphatase (AP) or horseradish peroxidase (HRP) or with fluorescent dyes. The method of producing these conjugates is determined by the reactive group on the label added.

Therapeutic Uses for Antibodies

The antibodies described above can be used to treat FGF-5 expressing or overexpressing tumors, for example by decreasing FGF-5 activity. Assays to determine whether an antibody modulates the immune response are described in EXAMPLES 2-4. Antibodies which recognize

FGF-5, may cause regression of an FGF-5 expressing or overexpressing tumor. In addition, antibodies can be used as diagnostic agents, for example to monitor the progression or regression of an FGF-5 expressing or over expressing tumor in a subject, for example a subject undergoing therapy to treat the neoplasim.

EXAMPLE 10

Diagnostic Methods

One embodiment disclosed herein is a method of pre-screening cells, such as tumor cells to determine if the cells express or over-express FGF-5, and if the subject is HLA-A3$^+$. This can be determined by conventional methods for identifying cells which present a particular HLA molecule, and identifying cells expressing DNA of the pertinent sequences, in this case a FGF-5 sequence. In one embodiment, once cells presenting the relevant HLA complex are identified via the foregoing screening methodology, they can be combined with a sample from a subject, where the sample contains CTLs. If the complex-presenting cells are lysed by the mixed CTL sample, then it can be assumed that a FGF-5 TAA is being presented, and the subject is an appropriate candidate for the therapeutic approaches set forth herein.

A subject can be screened to determine if the cells of a subject carry an FGF-5 gene, for example a wild-type FGF-5 gene, polymorphic, mutant or variant FGF-5 genes having a heterozygous or homozygous nucleotide change, or insertions, partial deletion, or duplications of the FGF-5 gene. One major application of the FGF-5 sequence information presented herein is in the area of genetic testing for predisposition to disease, such as cancer, owing to the presence and expression or overexpression of an FGF-5 gene. Predisposition to other diseases, including, but not limited to Hippel-Lindau disease, horseshoe kidneys, adult polycystic kidney disease, and acquired renal cystic disease, owing to the presence and expression or overexpression of an FGF-5 gene, can also be determined using the methods disclosed herein.

The sequence of FGF-5 genes, including intron-exon boundaries, is also useful in such diagnostic methods. The method consists of providing a biological sample obtained from the subject, in which sample includes DNA or RNA, and providing an assay for detecting in the biological sample the presence, absence, variant, or mutation of a FGF-5 gene and FGF-5 RNA. Suitable biological samples include samples obtained from body cells, such as those present in peripheral blood, urine, saliva, tissue biopsy, surgical specimen, fine needle aspirate specimen, amniocentesis samples and autopsy material. The detection in the biological sample may be performed by a number of methodologies, as outlined below.

The foregoing assay may be assembled in the form of a diagnostic kit and may include, for example, hybridization with oligonucleotides; PCR amplification of the gene or a part thereof using

oligonucleotide primers; RT-PCR amplification of the RNA or a part thereof using oligonucleotide primers; or direct sequencing of an FGF-5 gene of the subject's genome using oligonucleotide primers. The efficiency of these molecular genetic methods should permit a rapid classification of patients affected by the presence of one or more copies of an FGF-5 gene, or deletions, variants, polymorphisms or mutations of a FGF-5 gene.

One embodiment of such detection techniques is the PCR amplification of reverse transcribed RNA (RT-PCR) of RNA isolated from cells (for example cells from a tumor) followed by direct DNA sequence determination of the products. The presence of one or more nucleotide differences between the obtained sequence and the cDNA sequences, and especially, differences in the ORF portion of the nucleotide sequence are taken as indicative of a potential FGF-5 gene mutation.

Alternatively, DNA extracted from tumor or other cells may be used directly for amplification. The direct amplification from genomic DNA would be appropriate for analysis of the entire FGF-5 gene including regulatory sequences located upstream and downstream from the open reading frame. Reviews of direct DNA diagnosis have been presented by Caskey (*Science* 236:1223-8, 1989) and by Landegren *et al.* (*Science* 242:229-37, 1989).

Further studies of FGF-5 genes isolated from subjects may reveal particular mutations, duplications, variants, polymorphisms, or deletions, which occur at a high frequency within this population of individuals. In this case, rather than sequencing the entire FGF-5 gene, it is possible to design DNA diagnostic methods to specifically detect the most common FGF-5 mutations, variants, polymorphisms, or deletions.

The detection of specific DNA mutations may be achieved by methods such as hybridization using specific oligonucleotides (Wallace *et al.*, 1986, *Cold Spring Harbor Symp. Quant. Biol.* 51:257-61), direct DNA sequencing (Church and Gilbert, 1984, *Proc. Natl. Acad. Sci. USA*. 81:1991-5), the use of restriction enzymes (Flavell *et al.*, 1978, *Cell* 15:25; Geever *et al.*, 1981, *Proc. Natl. Acad. Sci USA* 78:5081), discrimination on the basis of electrophoretic mobility in gels with denaturing reagent (Myers and Maniatis, 1986, *Cold Spring Harbor Symp. Quant. Biol.* 51:275-84), RNase protection (Myers *et al.*, 1985, *Science* 230:1242), chemical cleavage (Cotton *et al.*, 1985, *Proc. Natl. Acad. Sci. USA* 85:4397-401), and the ligase-mediated detection procedure (Landegren *et al.*, 1988, *Science* 241:1077).

Oligonucleotides specific to normal, variant, polymorphic, or mutant sequences are chemically synthesized using commercially available machines, labeled radioactively with isotopes (such as ^{32}P) or non-radioactively, with tags such as biotin (Ward and Langer *et al.*, 1981, *Proc. Natl. Acad. Sci. USA* 78:6633-57), and hybridized to individual DNA samples immobilized on membranes or other solid supports by dot-blot or transfer from gels after electrophoresis. The

presence of these specific sequences are visualized by methods such as autoradiography or fluorometric (Landegren *et al.*, 1989, *Science* 242:229-37) or colorimetric reactions (Gebeyehu *et al.*, 1987, *Nucleic Acids Res.* 15:4513-34). The absence of hybridization would indicate a mutation in the particular region of the gene, or a deleted FGF-5 gene.

Sequence differences between normal, variant, polymorphic, and mutant forms of FGF-5 genes may also be revealed by the direct DNA sequencing method of Church and Gilbert (*Proc. Natl. Acad. Sci. USA* 81:1991-5, 1988). Cloned DNA segments may be used as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR (Wrichnik *et al.*, 1987, *Nucleic Acids Res.* 15:529-42; Wong *et al.*, 1987, *Nature* 330:384-6; Stoflet *et al.*, 1988, *Science* 239:491-4). In this approach, a sequencing primer which lies within the amplified sequence is used with double-stranded PCR product or single-stranded template generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotides or by automatic sequencing procedures with fluorescent tags.

Sequence alterations may occasionally generate fortuitous restriction enzyme recognition sites or may eliminate existing restriction sites. Changes in restriction sites are revealed by the use of appropriate enzyme digestion followed by conventional gel-blot hybridization (Southern, 1975, *J. Mol. Biol.* 98:503). DNA fragments carrying the site (either normal or mutant) are detected by their reduction in size or increase of corresponding restriction fragment numbers. Genomic DNA samples may also be amplified by PCR prior to treatment with the appropriate restriction enzyme; fragments of different sizes are then visualized under UV light in the presence of ethidium bromide after gel electrophoresis.

Genetic testing based on DNA sequence differences can be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing reagent. Small sequence deletions and insertions can be visualized by high-resolution gel electrophoresis. For example, a PCR product with small deletions is clearly distinguishable from a normal sequence on an 8% non-denaturing polyacrylamide gel (WO 91/10734; Nagamine *et al.*, 1989, *Am. J. Hum. Genet.* 45:337-9). DNA fragments of different sequence compositions may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific "partial-melting" temperatures (Myers *et al.*, 1985, *Science* 230:1242). Alternatively, a method of detecting a mutation comprising a single base substitution or other small change could be based on differential primer length in a PCR. For example, an invariant primer could be used in addition to a primer specific for a mutation. The PCR products of the normal and mutant genes can then be differentially detected in acrylamide gels.

In addition to conventional gel-electrophoresis and blot-hybridization methods, DNA fragments may also be visualized by methods where the individual DNA samples are not immobilized on membranes. The probe and target sequences may be both in solution, or the probe sequence may be immobilized (Saiki *et al.*, 1989, *Proc. Nat. Acad. Sci. USA* 86:6230-4). A variety of detection methods, such as autoradiography involving radioisotopes, direct detection of radioactive decay (in the presence or absence of scintillant), spectrophotometry involving calorigenic reactions and fluorometry involved fluorogenic reactions, may be used to identify specific individual genotypes.

If more than one mutation is frequently encountered in FGF-5 genes, a system capable of detecting such multiple mutations is desirable. For example, a PCR with multiple, specific oligonucleotide primers and hybridization probes may be used to identify all possible mutations at the same time (Chamberlain et al., 1988, *Nucl. Acids Res.* 16:1141-55). The procedure may involve immobilized sequence-specific oligonucleotides probes (Saiki et al., 1989, *Proc. Nat. Acad. Sci. USA* 86:6230-4).

One method particularly suitable for detecting the presence of FGF-5 genes disclosed herein is the use of high density oligonucleotide arrays (also known as "DNA chips") as described by Hacia *et al.* (*Nat. Genet.* 14:441-7, 1996).

EXAMPLE 11

Two Step Assay to Detect the Presence of FGF-5 gene in a Sample

A tissue sample from a subject can be processed according to the method disclosed by Antonarakis *et al.* (*New Eng. J. Med.* 313:842-848, 1985), separated through a 1% agarose gel and transferred to a nylon membrane for Southern blot analysis. Membranes are UV cross linked at 150 mJ using a GS Gene Linker (Bio-Rad, Hercules, CA). An FGF-5 probe is subcloned into pTZ18U. The phagemids can be transformed into *E. coli* MV 1190 infected with M13KO7 helper phage (Bio-Rad, Richmond, Calif.). Single stranded DNA is isolated according to standard procedures (Sambrook, *et al. Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989).

Blots are prehybridized for 15-30 minutes at 65°C in 7% sodium dodecyl sulfate (SDS) in 0.5 M $NaPO_4$. The methods follow those described by Nguyen, et al. (*BioTechniques* 13:116-123, 1992). The blots are hybridized overnight at 65°C in 7% SDS, 0.5 M $NaPO_4$ with 25-50 ng/ml single stranded probe DNA. Post-hybridization washes consist of two 30 minute washes in 5% SDS, 40 mM $NaPO_4$ at 65°C, followed by two 30-minute washes in 1% SDS, 40 mM $NaPO_4$ at 65°C.

The blots are subsequently rinsed with phosphate buffered saline (pH 6.8) for five minutes at RT and incubated with 0.2% casein in PBS for five minutes. The blots are then preincubated for 5-10 minutes in a shaking water bath at 45°C with hybridization buffer consisting of 6 M urea, 0.3 M NaCl, and 5X Denhardt's solution (see Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989). The buffer is removed and replaced with 50-75 $\mu l/cm^2$ fresh hybridization buffer plus 2.5 nM of the covalently cross-linked oligonucleotide sequence complementary to the universal primer site (UP-AP, Bio-Rad). The blots are hybridized for 20-30 minutes at 45°C and post hybridization washes are incubated at 45°C as two 10 minute washes in 6 M urea, 1X standard saline citrate (SSC), 0.1% SDS and one 10 minute wash in 1XSSC, 0.1% Triton™X-100. The blots are rinsed for 10 minutes at RT with 1XSSC.

Blots are incubated for 10 minutes at RT with shaking in the substrate buffer consisting of 0.1 M diethanolarnine, 1 mM $MgCl_2$, 0.02% sodium azide, pH 10.0. Individual blots are placed in heat sealable bags with substrate buffer and 0.2 mM AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3'-phosphoryloxy)phenyl-1,2-dioxetane, disodium salt, Bio-Rad). After a 20 minute incubation at RT with shaking, the excess AMPPD solution is removed. The blot is exposed to X-ray film overnight. Patient samples which show no hybridizing bands lack a FGF-5 gene. Patient samples which show positive bands indicate the presence of at least one copy of an FGF-5 gene, possibly more, which would indicate the possibility of ongoing disease such as cancer, such as RCC, prostate or breast cancer, or an enhanced susceptibility to developing a disease, such as cancer, in the future.

In other embodiments, the method involves assaying for the presence of transcription factors which may modulate expression of FGF-5. Patient samples which show positive bands for the transcription factor indicate the presence of at least one copy of the transcription factor gene, possibly more, which would indicate the possibility of ongoing disease such as cancer, such as RCC, prostate or breast cancer, or an enhanced susceptibility to developing a disease, such as cancer, in the future.

EXAMPLE 12

Quantitation of FGF-5 Proteins

An alternative method of diagnosing the presence of an FGF-5 and/or transcription factor gene is to quantitate the level of FGF-5 protein in the cells of a subject. This diagnostic tool is useful for detecting elevated levels of FGF-5 protein which result from, for example, expression or overexpression of the FGF-5 gene, the presence of multiple copies of an FGF-5 and/or transcription factor(s) gene, or mutations (such as mutations within the gene, or mutations upstream or downstream from the gene which affect its expression) which result in increased expression of

FGF-5. These diagnostic methods, in addition to those described in EXAMPLES 10 and 11, provide an enhanced ability to diagnose susceptibility to diseases caused by FGF-5 expression or overexpression.

The determination of elevated FGF-5 protein levels is an alternative or supplemental approach to the direct determination of whether the FGF-5 gene (or transcription factor gene(s)) is present in multiple copies or if mutations are present (for example mutations up- or down-stream of an FGF-5 gene) that would result in increased FGF-5 expression, using the methods outlined above in EXAMPLES 10 and 11. The availability of antibodies specific to the FGF-5 protein (for example those described in EXAMPLE 9) will facilitate the quantitation of cellular FGF-5 protein by one of a number of immunoassay methods which are well known in the art and are presented in Harlow and Lane (*Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, New York. 1988).

Such assays permit both the detection of FGF-5 proteins in a biological sample and the quantitation of such proteins. Typical methods involve providing a biological sample of the subject in which the sample contains cellular proteins, and providing an immunoassay for quantitating the level of FGF-5 protein in the biological sample. This can be achieved by combining the biological sample with a FGF-5 specific binding agent, such as an anti-FGF-5 antibody (such as monoclonal or polyclonal antibodies), so that complexes form between the binding agent and the FGF-5 protein present in the sample, and then detecting or quantitating such complexes.

In particular forms, these assays may be performed with the FGF-5 specific binding agent immobilized on a support surface, such as in the wells of a microtiter plate or on a column. The biological sample is then introduced onto the support surface and allowed to interact with the specific binding agent so as to form complexes. Excess biological sample is then removed by washing, and the complexes are detected with a reagent, such as a second anti-FGF-5 protein antibody that is conjugated with a detectable marker.

In an alternative assay, the cellular proteins are isolated and subjected to SDS-PAGE followed by Western blotting. After resolving the proteins, the proteins are transferred to a membrane, which is probed with specific binding agents that recognize FGF-5. The proteins are detected, for example with HRP-conjugated secondary antibodies, and quantitated.

In yet another assay, the level of FGF-5 protein in cells is analyzed using microscopy. Using specific binding agents which recognize FGF-5, samples can be analyzed for the presence of FGF-5 proteins. For example, frozen biopsied tissue sections are thawed at room temperature and fixed with acetone at -200°C for five minutes. Slides are washed twice in cold PBS for five minutes each, then air-dried. Sections are covered with 20-30 μl of antibody solution (15-45 μg/ml) (diluted in PBS, 2% BSA at 15-50 μg/ml) and incubated at RT in a humidified chamber for

30 minutes. Slides are washed three times with cold PBS five minutes each, allowed to air-dry briefly (5 minutes) before applying 20-30 μl of the second antibody solution (diluted in PBS, 2% BSA at 15-50 μg/ml) and incubated at RT in humidified chamber for 30 minutes. The label on the second antibody may contain a fluorescent probe, enzyme, radiolabel, biotin, or other detectable marker. The slides are washed three times with cold PBS five minutes each then quickly dipped in distilled water, air-dried, and mounted with PBS containing 30% glycerol. Slides can be stored at 4°C prior to viewing.

For samples prepared for electron microscopy (versus light microscopy), the second antibody is conjugated to gold particles. Tissue is fixed and embedded with epoxy plastics, then cut into very thin sections (~1-2 μm). The specimen is then applied to a metal grid, which is then incubated in the primary anti-FGF-5 antibody, washed in a buffer containing BSA, then incubated in a secondary antibody conjugated to gold particles (usually 5-20 nm). These gold particles are visualized using electron microscopy methods.

For the purposes of quantitating FGF-5 proteins in a sample specimen, a "control" sample of the subject, which sample does not express FGF-5 or expresses FGF-5 at lower levels, can be used to compare FGF-5 expression in the sample to be analyzed. Such a "control" sample may be obtained from normal adult cells, such as those present in which expression of the protein is not detected. As shown in FIG. 4, for example, FGF-5 is not expressed in detectable levels in any normal tissue tested. However, peripheral blood leukocytes is clearly the most accessible and convenient source from which comparative samples can be obtained. Sample specimens can be obtained from peripheral blood, urine, saliva, tissue biopsy, amniocentesis samples, surgical specimens, fine needle aspirates, and autopsy material, particularly cancer cells. Quantitation of FGF-5 proteins can be made by immunoassay and compared to levels of the protein found in non-FGF-5 expressing cells, such as normal adult tissues, or to the level of FGF-5 in healthy, normal cells (for example, cells of the same origin that are not neoplastic or are free of the disease of interest). A significant (for example 50% or greater) increase in the amount of FGF-5 protein in the cells of a subject compared to the amount of FGF-5 protein found in non-FGF-5 expressing cells or that found in normal cells, would be taken as an indication that the presence of the FGF-5 gene locus in the subject.

EXAMPLE 13

Gene Therapy

A new gene therapy approach for subjects suffering from tumors which express or over-express FGF-5 is now made possible by the present disclosure. Administration of FGF-5 to a subject suffering from a tumor that expresses or over-expresses FGF-5, may further enhance the

modulation of the immune response, such as stimulating CTLs to lyse the FGF-5 expressing tumor cells, and achieve the desired therapeutic goal of assisting in regression of the tumor. In some subjects, expression or overexpression of FGF-5 by the tumor may be insufficient to stimulate the immune system, for example in non HLA-A3 subjects. Alternatively, modulation of the immune response may be augmented by additional FGF-5 expression. Essentially, cells may be removed from a subject, such as dendritic cells, suffering from a tumor that expresses or over-expresses FGF-5, and then transfected with an expression vector containing FGF-5 cDNA. These transfected cells will thereby produce functional FGF-5 protein and can be reintroduced into the subject to stimulate CTLs.

The scientific and medical procedures required for human cell transfection are now routine procedures. The provision herein of FGF-5 cDNAs now allows the development of human gene therapy based upon these procedures. Immunotherapy of melanoma patients using genetically engineered tumor-infiltrating lymphocytes (TILs) has been reported by Rosenberg *et al.* (*N. Engl. J. Med.* 323:570-8, 1990). In that study, a retrovirus vector was used to introduce a gene for neomycin resistance into TILs. A similar approach may be used to introduce the FGF-5 cDNA into patients affected by FGF-5 expression or overexpression.

In some embodiments, a method of treating tumors which express or overexpress FGF-5, or in which greater FGF-5 expression is desired, is disclosed. These methods can be accomplished by introducing a gene coding for FGF-5 into a subject. A general strategy for transferring genes into donor cells is disclosed in U.S. Patent No. 5,529,774. Generally, a gene encoding a protein having therapeutically desired effects is cloned into a viral expression vector, and that vector is then introduced into the target organism. The virus infects the cells, and produces the protein sequence *in vivo*, where it has its desired therapeutic effect. See, for example, Zabner *et al.* (*Cell* 75:207-16, 1993).

In some of the foregoing examples, it may only be necessary to introduce the genetic or protein elements into certain cells or tissues. For example, in the case of benign nevi and psoriasis, introducing them into only the skin may be sufficient. However, in some instances (i.e. tumors), it may be more therapeutically effective and simple to treat all of the patients cells, or more broadly disseminate the vector, for example by intravascular administration.

The nucleic acid sequence encoding at least one therapeutic agent is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, the gene's native promoter, retroviral LTR promoter, or adenoviral promoters, such as the adenoviral major late promoter; the cytomegalovirus (CMV) promoter; the Rous Sarcoma Virus (RSV) promoter; inducible promoters, such as the MMTV promoter; the metallothionein promoter; heat shock promoters; the albumin promoter; the histone promoter; the α-actin promoter; TK

promoters; B19 parvovirus promoters; and the ApoAI promoter. However the scope of the disclosure is not limited to specific foreign genes or promoters.

The recombinant nucleic acid can be administered to the subject by any method which allows the recombinant nucleic acid to reach the appropriate cells. These methods include injection, infusion, deposition, implantation, or topical administration. Injections can be intradermal or subcutaneous. The recombinant nucleic acid can be delivered as part of a viral vector, such as avipox viruses, recombinant vaccinia virus, replication-deficient adenovirus strains or poliovirus, or as a non-infectious form such as naked DNA or liposome encapsulated DNA.

EXAMPLE 14

Viral Vectors for Gene Therapy

Adenoviral vectors may include essentially the complete adenoviral genome (Shenk *et al.*, *Curr. Top. Microbiol. Immunol.* 111:1-39, 1984). Alternatively, the adenoviral vector may be a modified adenoviral vector in which at least a portion of the adenoviral genome has been deleted. In one embodiment, the vector includes an adenoviral 5' ITR; an adenoviral 3' ITR; an adenoviral encapsidation signal; a DNA sequence encoding a therapeutic agent; and a promoter for expressing the DNA sequence encoding a therapeutic agent. The vector is free of at least the majority of adenoviral E1 and E3 DNA sequences, but is not necessarily free of all of the E2 and E4 DNA sequences, and DNA sequences encoding adenoviral proteins transcribed by the adenoviral major late promoter. In another embodiment, the vector may be an adeno-associated virus (AAV) such as described in U.S. Patent No. 4,797,368 (Carter *et al.*) and in McLaughlin *et al.* (*J. Virol.* 62:1963-73, 1988) and AAV type 4 (Chiorini *et al. J. Virol.* 71:6823-33, 1997) and AAV type 5 (Chiorini *et al. J. Virol.* 73:1309-19, 1999).

Such a vector may be constructed according to standard techniques, using a shuttle plasmid which contains, beginning at the 5' end, an adenoviral 5' ITR, an adenoviral encapsidation signal, and an E1a enhancer sequence; a promoter (which may be an adenoviral promoter or a foreign promoter); a tripartite leader sequence, a multiple cloning site (which may be as herein described); a poly A signal; and a DNA segment which corresponds to a segment of the adenoviral genome. The DNA segment serves as a substrate for homologous recombination with a modified or mutated adenovirus, and may encompass, for example, a segment of the adenovirus 5' genome no longer than from base 3329 to base 6246. The plasmid may also include a selectable marker and an origin of replication. The origin of replication may be a bacterial origin of replication. A desired DNA sequence encoding a therapeutic agent may be inserted into the multiple cloning site of the plasmid.

The plasmid may be used to produce an adenoviral vector by homologous recombination with a modified or mutated adenovirus in which at least the majority of the E1 and E3 adenoviral

DNA sequences have been deleted. Homologous recombination may be effected through co-transfection of the plasmid vector and the modified adenovirus into a helper cell line, such as 293 cells, by $CaPO_4$ precipitation. The homologous recombination produces a recombinant adenoviral vector which includes DNA sequences derived from the shuttle plasmid between the Not I site and the homologous recombination fragment, and DNA derived from the E1 and E3 deleted adenovirus between the homologous recombination fragment and the 3' ITR.

In one embodiment, the adenovirus may be constructed by using a yeast artificial chromosome (or YAC) containing an adenoviral genome according to the method described in Ketner *et al.* (*Proc. Natl. Acad. Sci. USA*, 91:6186-90, 1994), in conjunction with the teachings contained herein. In this embodiment, the adenovirus yeast artificial chromosome is produced by homologous recombination *in vivo* between adenoviral DNA and yeast artificial chromosome plasmid vectors carrying segments of the adenoviral left and right genomic termini. A DNA sequence encoding a therapeutic agent then may be cloned into the adenoviral DNA. The modified adenoviral genome then is excised from the adenovirus yeast artificial chromosome in order to be used to generate adenoviral vector particles as hereinabove described.

The adenoviral particles are administered in an amount effective to produce a therapeutic effect in a subject. The exact dosage of adenoviral particles to be administered is dependent upon a variety of factors, including the age, weight, and sex of the subject to be treated, and the nature and extent of the disease or disorder to be treated. The adenoviral particles may be administered as part of a preparation having a titer of adenoviral particles of at least 1×10^{10} pfu/ml, and in general not exceeding 2×10^{11} pfu/ml. The adenoviral particles may be administered in combination with a pharmaceutically acceptable carrier in a volume up to 10 ml. The pharmaceutically acceptable carrier may be, for example, a liquid carrier such as a saline solution, protamine sulfate (Elkins-Sinn, Inc., Cherry Hill, NJ), or Polybrene (Sigma Chemical) as well as those described in EXAMPLE 18.

In another embodiment, the viral vector is a retroviral vector. Retroviruses have been considered for experiments in gene therapy because they have a high efficiency of infection and stable integration and expression (Orkin *et al.*, 1988, *Prog. Med. Genet.*7:130-42). Partial or full length FGF-5 genes can be cloned into a retroviral vector and driven from either its endogenous promoter or from the retroviral LTR (long terminal repeat). Examples of retroviral vectors which may be employed include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus. The vector is generally a replication defective retrovirus particle.

Retroviral vectors are useful as agents to effect retroviral-mediated gene transfer into eukaryotic cells. Retroviral vectors are generally constructed such that the majority of sequences coding for the structural genes of the virus are deleted and replaced by the gene(s) of interest. Most often, the structural genes (i.e., gag, pol, and env) are removed from the retroviral backbone using genetic engineering techniques known in the art. This may include digestion with the appropriate restriction endonuclease or, in some instances, with Bal 31 exonuclease to generate fragments containing appropriate portions of the packaging signal.

Other viral transfection systems may also be utilized for this type of approach, including Vaccinia virus (Moss *et al.*, 1987, *Annu. Rev. Immunol.* 5:305-24), Bovine Papilloma virus (Rasmussen *et al.*, 1987, *Methods Enzymol.* 139:642-54) or members of the herpes virus group such as Epstein-Barr virus (Margolskee *et al.*, 1988, *Mol. Cell. Biol.* 8:2837-47). Recent developments in gene therapy techniques include the use of RNA-DNA hybrid oligonucleotides, as described by Cole-Strauss *et al.* (*Science* 273:1386-9, 1996). This technique can allow for site-specific integration of cloned sequences, permitting accurately targeted gene replacement.

New genes may be incorporated into proviral backbones in several general ways. In the most straightforward constructions, the structural genes of the retrovirus are replaced by a single gene which then is transcribed under the control of the viral regulatory sequences within the long terminal repeat (LTR). Retroviral vectors have also been constructed which can introduce more than one gene into target cells. Usually, in such vectors one gene is under the regulatory control of the viral LTR, while the second gene is expressed either off a spliced message or is under the regulation of its own, internal promoter. Alternatively, two genes may be expressed from a single promoter by the use of an Internal Ribosome Entry Site.

EXAMPLE 15

Peptide Modifications

The present disclosure includes biologically active molecules that mimic the action (mimetics) of the FGF-5 proteins disclosed herein. The disclosure therefore includes synthetic embodiments of naturally-occurring peptides, as well as analogues (non-peptide organic molecules), derivatives (chemically functionalized peptide molecules obtained starting with the disclosed peptide sequences) and variants (homologs) of these peptides that stimulate an HLA-A3-restricted CTL immune response against FGF-5 expressing or over-expressing tumors, as measured by IFN-γ concentration. Each peptide ligand disclosed is comprised of a sequence of amino acids, which may be either L- and/or D- amino acids, naturally occurring and otherwise.

Peptides may be modified by a variety of chemical techniques to produce derivatives having essentially the same activity as the unmodified peptides, and optionally having other

desirable properties. For example, carboxylic acid groups of the peptide, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C1-C16 ester, or converted to an amide of formula NR1R2 wherein R1 and R2 are each independently H or C1-C16 alkyl, or combined to form a heterocyclic ring, such as a 5- or 6- membered ring. Amino groups of the peptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be modified to C1-C16 alkyl or dialkyl amino or further converted to an amide.

Hydroxyl groups of the peptide side chain may be converted to C1-C16 alkoxy or to a C1-C16 ester using well-recognized techniques. Phenyl and phenolic rings of the peptide side chain may be substituted with one or more halogen atoms, such as fluorine, chlorine, bromine or iodine, or with C1-C16 alkyl, C1-C16 alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the peptide sidechains can be extended to homologous C2-C4 alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the peptides disclosed herein to select and provide conformational constraints to the structure that result in enhanced stability. For example, a carboxyl-terminal or amino-terminal cysteine residue can be added to the peptide, so that when oxidized the peptide will contain a disulfide bond, thereby generating a cyclic peptide. Other peptide cyclizing methods include the formation of thioethers and carboxyl- and amino-terminal amides and esters.

To maintain an optimally functional peptide, particular peptide variants will differ by only a small number of amino acids from the peptides disclosed herein. Such variants may have deletions (for example of 1-3 or more amino acid residues), insertions (for example of 1-3 or more residues), or substitutions that do not interfere with the desired activity of the peptides. Substitutional variants are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. In particular embodiments, such variants will have amino acid substitutions of single residues, for example 1, 3, 5 or even 10 substitutions in the FGF-5 protein (SEQ ID NOS: 4, 6, 8, 10, 12, and 16-19).

Peptidomimetic and organomimetic embodiments are also disclosed, whereby the three-dimensional arrangement of the chemical constituents of such peptido- and organomimetics mimic the three-dimensional arrangement of the peptide backbone and component amino acid side chains in the peptide, resulting in such peptido- and organomimetics of the peptides having the ability to modulate an immune response against FGF-5 expressing or over-expressing tumors, as measured by IFN-γ concentration (FGF-5 mimetics). For computer modeling applications, a pharmacophore is an idealized, three-dimensional definition of the structural requirements for modulating an

immune response activity. Peptido- and organomimetics can be designed to fit each pharmacophore with current computer modeling software (using computer assisted drug design or CADD). See Walters, "Computer-Assisted Modeling of Drugs", in Klegerman & Groves, eds., 1993, Pharmaceutical Biotechnology, Interpharm Press: Buffalo Grove, IL, pp. 165-174 and Principles of Pharmacology (ed. Munson, 1995), chapter 102 for a description of techniques used in CADD. Also disclosed are mimetics prepared using such techniques that produce either peptides or conventional organic pharmaceuticals that retain the ability to modulate an immune response or modulate FGF-5 expression or activity.

The above described mimetics are examined for their ability to stimulate an HLA-A3-restricted CTL immune response against FGF-5 expressing or over-expressing tumors, as measured by IFN-γ concentration. Such activities can be readily determined using the assays disclosed herein, for example using the methods described in EXAMPLES 2-4. Suitable mimetics would demonstrate the ability to modulate an immune response or modulate FGF-5 expression or activity as defined above.

EXAMPLE 16

Methods for Generating Mimetics

Compounds or other molecules which mimic normal FGF-5 function, such as compounds which modulate an immune response or modulate FGF-5 expression or activity, for example modulate an immune response against FGF-5 expressing or over-expressing tumors, as measured by IFN-γ concentration, can be identified and/or designed. These compounds or molecules are known as mimetics, because they mimic the biological activity of the normal protein.

Crystallography

To identify the amino acids that interact between FGF-5 and the MHC of the HLA-A3-restricted CTLs (the MHC), FGF-5 is co-crystallized in the presence of the MHC. One method that can be used is the hanging drop method. In this method, a concentrated salt, the FGF-5 protein and the MHC protein solution is applied to the underside of a lid of a multiwell dish. A range of concentrations may need to be tested. The lid is placed onto the dish, such that the droplet "hangs" from the lid. As the solvent evaporates, a protein crystal is formed, which can be visualized with a microscope. This crystallized structure is then subjected to X-ray diffraction or NMR analysis which allows for the identification of the amino acid residues that are in contact with one another. The amino acids that contact the transcription factors establish a pharmacophore that can then be used to identify drugs that interact at that same site.

Identification of drugs

Once these amino acids have been identified, one can screen synthetic drug databases (which can be licensed from several different drug companies), to identify drugs that interact with the same amino acids of a FGF-5 protein that the MHC interacts with. Moreover, structure activity relationships and computer assisted drug design can be performed as described in Remington, *The Science and Practice of Pharmacy*, Chapter 28.

Designing synthetic peptides

In addition, synthetic peptides can be designed from the sequence of the MHC that interacts with FGF-5. Several different peptides could be generated from this region(s). This could be done with or without the crystallography data. However, once crystallography data is available, peptides can also be designed that bind better than FGF-5.

The chimeric peptides may be expressed recombinantly, for example in *E. coli*. One advantage of the synthetic peptides over the monoclonal antibodies is that they are smaller, and therefore diffuse easier, and are not as likely to be immunogenic. Standard mutagenesis of such peptides can also be performed to identify variant peptides having even greater ability to modulate an immune response or modulate FGF-5 expression or activity as defined herein.

After synthetic drugs or peptides that bind to FGF-5 have been identified, their ability to stimulate an HLA-A3-restricted CTL immune response against FGF-5 expressing or over-expressing tumors, as measured by IFN-γ concentration, can be tested as described in EXAMPLES 2-4. Those that are positive would be good candidates for therapies, such as treatment of tumors in which FGF-5 is expressed or overexpressed.

EXAMPLE 17

Peptide Synthesis and Purification

The disclosed peptides (and variants and fragments thereof) can be chemically synthesized by any of a number of manual or automated methods of synthesis known in the art. For example, solid phase peptide synthesis (SPPS) is carried out on a 0.25 millimole (mmole) scale using an Applied Biosystems Model 431A Peptide Synthesizer and using 9-fluorenylmethyloxycarbonyl (Fmoc) amino-terminus protection, coupling with dicyclohexylcarbodiimide/ hydroxybenzotriazole or 2-(1H-benzo-triazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/ hydroxybenzotriazole (HBTU/HOBT), and using p-hydroxymethylphenoxymethylpolystyrene (HMP) or Sasrin resin for carboxyl-terminus acids or Rink amide resin for carboxyl-terminus amides.

Fmoc-derivatized amino acids are prepared from the appropriate precursor amino acids by tritylation and triphenylmethanol in trifluoroacetic acid, followed by Fmoc derivitization as described by Atherton *et al.* (*Solid Phase Peptide Synthesis*, IRL Press: Oxford, 1989).

Sasrin resin-bound peptides are cleaved using a solution of 1% TFA in dichloromethane to yield the protected peptide. Where appropriate, protected peptide precursors are cyclized between the amino- and carboxyl-termini by reaction of the amino-terminal free amine and carboxyl-terminal free acid using diphenylphosphorylazide in nascent peptides wherein the amino acid sidechains are protected.

HMP or Rink amide resin-bound products are routinely cleaved and protected sidechain-containing cyclized peptides deprotected using a solution comprised of trifluoroacetic acid (TFA), optionally also comprising water, thioanisole, and ethanedithiol, in ratios of 100 : 5 : 5 : 2.5, for 0.5 - 3 hours at RT.

Crude peptides are purified by preparative high pressure liquid chromatography (HPLC), for example using a Waters Delta-Pak C18 column and gradient elution with 0.1% TFA in water modified with acetonitrile. After column elution, acetonitrile is evaporated from the eluted fractions, which are then lyophilized. The identity of each product so produced and purified may be confirmed by fast atom bombardment mass spectroscopy (FABMS) or electrospray mass spectroscopy (ESMS).

EXAMPLE 18

Pharmaceutical Compositions and Modes of Administration

Various delivery systems for administering the combined therapy disclosed herein are known, and include e.g., encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis (see Wu and Wu, *J. Biol. Chem.* 1987, 262:4429-32), and construction of a therapeutic nucleic acid as part of a retroviral or other vector. Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, the pharmaceutical compositions may be introduced into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

In one embodiment, it may be desirable to administer the pharmaceutical compositions disclosed herein locally to the area in need of treatment, for example, by local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, through a catheter, by a suppository or an implant, such as a porous, non-porous, or gelatinous material, including membranes, such as silastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

The use of liposomes as a delivery vehicle is one delivery method of interest. The liposomes fuse with the target site and deliver the contents of the lumen intracellularly. The liposomes are maintained in contact with the target cells for a sufficient time for fusion to occur, using various means to maintain contact, such as isolation and binding agents. Liposomes may be prepared with purified proteins or peptides that mediate fusion of membranes, such as Sendai virus or influenza virus. The lipids may be any useful combination of known liposome forming lipids, including cationic lipids, such as phosphatidylcholine. Other potential lipids include neutral lipids, such as cholesterol, phosphatidyl serine, phosphatidyl glycerol, and the like. For preparing the liposomes, the procedure described by Kato *et al.* (*J. Biol. Chem.* 1991, 266:3361) may be used.

The present disclosure also provides pharmaceutical compositions which include a therapeutically effective amount of autologous CTLs specific to FGF-5 and/or an FGF-5 protein, RNA, DNA, antisense molecule or specific binding agent (for example, antibodies), alone or with a pharmaceutically acceptable carrier. In one example, homogeneous compositions of the FGF-5 therapeutic molecules includes compositions that are comprised of at least 90% of the peptide, variant, analog, derivative or mimetic in the composition.

Delivery systems

Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition can be sterile, and the formulation suits the mode of administration. The composition can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, and magnesium carbonate.

The amount of the therapeutic agent that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined

by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The disclosure also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Instructions for use of the composition can also be included.

The pharmaceutical compositions or methods of treatment may be administered in combination with other therapeutic treatments, such as other antineoplastic or antitumorigenic therapies.

Administration of Nucleic Acid Molecules

In an embodiment in which an FGF-5 nucleic acid is employed for gene therapy, the analog is delivered intracellularly (e.g., by expression from a nucleic acid vector or by receptor-mediated mechanisms). In an embodiment where the therapeutic molecule is a nucleic acid or antisense molecule, administration may be achieved by an appropriate nucleic acid expression vector which is administered so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA* 1991, 88:1864-8). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within a cell's cellular DNA for expression, by homologous recombination.

The vector pcDNA, is an example of a method of introducing the foreign cDNA into a cell under the control of a strong viral promoter (CMV) to drive the expression. However, other vectors can be used (see EXAMPLES 8 and 14). Other retroviral vectors (such as pRETRO-ON, Clontech), also use this promoter but have the advantages of entering cells without any transfection aid, integrating into the genome of target cells only when the target cell is dividing (as cancer cells do, especially during first remissions after chemotherapy) and they are regulated. It is also possible to turn on the expression of the FGF-5 nucleic acid by administering tetracycline when these

plasmids are used. Hence these plasmids can be allowed to transfect the cells, then administer a course of tetracycline with a course of chemotherapy to achieve better cytotoxicity.

Other plasmid vectors, such as pMAM-neo (Clontech) or pMSG (Amersham Pharmacia Biotech) use the MMTV-LTR promoter (which can be regulated with steroids) or the SV10 late promoter (pSVL, Amersham Pharmacia Biotech) or metallothionein - responsive promoter (pBPV, Amersham Pharmacia Biotech) and other viral vectors, including retroviruses. Examples of other viral vectors include adenovirus, AAV (adeno-associated virus), recombinant HSV, poxviruses (vaccinia) and recombinant lentivirus (such as HIV). These vectors achieve the basic goal of delivering into the target cell the cDNA sequence and control elements needed for transcription. The present disclosure includes all forms of nucleic acid delivery, including synthetic oligos, naked DNA, plasmid and viral, integrated into the genome or not.

Administration of Antibodies

In an embodiment where the therapeutic molecule is an antibody, specifically an antibody that recognizes FGF-5 proteins, administration may be achieved by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents. Similar methods can be used to administer FGF-5 proteins, of fragments thereof.

The present disclosure also provides pharmaceutical compositions which include a therapeutically effective amount of the antibody, and a pharmaceutically acceptable carrier or excipient.

EXAMPLE 19

Antisense Disruption of FGF-5 Expression

This example describes methods that can be used to decrease FGF-5 expression. Such methods are useful when treatment of tumors which express or over-express FGF-5 is desired, for example in RCC and carcinoma of the breast, prostate, bladder or pancreas. For example, some subjects may not respond to modulation of the immune response by an agent having FGF-5 activity. Such subjects may include non-HLA-A3 subjects. As an alternative, or additional, therapy, subjects having an FGF-5 expressing or overexpressing neoplasm may respond to therapies which modulate FGF-5 expression or activity, for example by decreasing any neoplastic response to FGF-5 expression, for example angiogenesis. One approach to decreasing FGF-5 expression, activity, or function, is to use antisense oligonucleotides.

To design an antisense oligonucleotide, the mRNA sequence from the desired molecule, such as human FGF-5, is examined. Regions of the sequence containing multiple repeats, such as

TTTTTTTT, are not as desirable because they will lack specificity. Several different regions can be chosen. Of those, oligos are selected by the following characteristics: ones having the best conformation in solution; ones optimized for hybridization characteristics; and one having less potential to form secondary structures. Antisense molecules having a propensity to generate secondary structures are less desirable.

Plasmids containing FGF-5 antisense sequences can also be genereated. For example, cDNA fragments coding for human FGF-5 are PCR amplified. The nücleotides are then amplified using Pfu DNA polymerase (Stratagene) and cloned in antisense orientation a vector, such as pcDNA vectors (InVitrogen). The nucleotide sequence and orientation of the insert can be confirmed by dideoxy sequencing using a Sequenase kit (Amersham Pharmacia Biotech).

Generally, the term "antisense" refers to a nucleic acid capable of hybridizing to a portion of an FGF-5 RNA (such as mRNA) by virtue of some sequence complementarity. The antisense nucleic acids disclosed herein can be oligonucleotides that are double-stranded or single-stranded, RNA or DNA or a modification or derivative thereof, which can be directly administered to a cell, or which can be produced intracellularly by transcription of exogenous, introduced sequences.

The FGF-5 antisense nucleic acids are polynucleotides, and may be oligonucleotides (ranging from 6 to about 100 oligonucleotides). In specific aspects, the oligonucleotide is at least 10, 15, 20, 62, 65, or 100 nucleotides, or a polynucleotide of at least 200 nucleotides. The antisense nucleic acids may be much longer constructs. The nucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The nucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, and may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA* 1989, 86:6553-6; Lemaitre et al., *Proc. Natl. Acad. Sci. USA* 1987, 84:648-52; PCT Publication No. WO 88/09810) or blood-brain barrier (see, e.g., PCT Publication No. WO 89/10134), hybridization triggered cleavage agents (see, e.g., Krol *et al.*, *BioTechniques* 1988, 6:958-76) or intercalating agents (see, e.g., Zon, *Pharm. Res.* 1988, 5:539-49).

In one embodiment disclosed herein, an FGF-5 antisense polynucleotide (including oligonucleotides) is provided, for example of single-stranded DNA. The FGF-5 antisense polynucleotide may recognize any species of FGF-5. The antisense polynucleotide may be modified at any position on its structure with substituents generally known in the art. For example, a modified base moiety may be 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N⁻6-sopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-

dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, methoxyarninomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-S-oxyacetic acid, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine.

In another embodiment, the polynucleotide includes at least one modified sugar moiety such as arabinose, 2-fluoroarabinose, xylose, and hexose, or a modified component of the phosphate backbone, such as phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, or a formacetal or analog thereof.

In yet another embodiment, the polynucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier *et al., Nucl. Acids Res.* 1987, 15:6625-41). The oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent. Oligonucleotides may include a targeting moiety that enhances uptake of the molecule by tumor cells. The targeting moiety can be a specific binding molecule, such as an antibody or fragment thereof that recognizes a molecule present on the surface of a diseased cell, such as a tumor cell.

As an alternative to antisense inhibitors, catalytic nucleic acid compounds, such as ribozymes or anti-sense conjugates, can be used to inhibit gene expression. Ribozymes may be synthesized and administered to the subject, or may be encoded on an expression vector, from which the ribozyme is synthesized in the targeted cell (as in PCT publication WO 9523225, and Beigelman *et al. Nucl. Acids Res.* 1995, 23:4434-42). Examples of oligonucleotides with catalytic activity are described in WO 9506764. Conjugates of antisense with a metal complex, e.g. terpyridylCu (II), capable of mediating mRNA hydrolysis, are described in Bashkin *et al.*, 1995, *Appl. Biochem Biotechnol.* 54:43-56.

Polynucleotides disclosed herein can be synthesized by standard methods known in the art, for example by use of an automated DNA synthesizer (Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligos may be synthesized by the method of Stein et al. (*Nucl. Acids Res.* 1998, 16:3209), methylphosphonate oligos can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:7448-51). In a specific embodiment, the ILP-2 and ILP-3 antisense oligonucleotides comprise catalytic RNA, or a

ribozyme (see PCT International Publication WO 90/11364, Sarver et al., *Science* 1990, 247:1222-5). In another embodiment, the oligonucleotide is a 2'-0-methylribonucleotide (Inoue et al., *Nucl. Acids Res.* 1987, 15:6131-48), or a chimeric RNA-DNA analogue (Inoue et al., *FEBS Lett.* 1987, 215:327-330).

The antisense polynucleic acids disclosed herein comprise a sequence complementary to at least a portion of an RNA transcript of an FGF-5 gene, such as a human FGF-5 gene. However, absolute complementarity, although advantageous, is not required. A sequence can be complementary to at least a portion of an RNA, meaning a sequence having sufficient complementarily to be able to hybridize with the RNA, forming a stable duplex; in the case of double-strandedFGF-5antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a FGF-5 RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

The relative ability of polynucleotides (such as oligonucleotides) to bind to complementary strands is compared by determining the melting temperature of a hybridization complex of the poly/oligonucleotide and its complementary strand. Base stacking, which occurs during hybridization, is accompanied by a reduction in UV absorption (hypochromicity). A reduction in UV absorption indicates a higher T_m. The higher the T_m the greater the strength of the binding of the hybridized strands. As close to optimal fidelity of base pairing as possible achieves optimal hybridization of a poly/oligonucleotide to its target RNA.

The amount of FGF-5 antisense nucleic acid which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In one embodiment, pharmaceutical compositions comprising FGF-5 antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In other embodiments, it may be useful to use such compositions to achieve sustained release of the FGF-5 antisense nucleic acids. In yet other embodiments, it may be desirable to utilize liposomes targeted via antibodies to specific identifiable tumor antigens (Leonetti *et al. Proc. Natl. Acad. Sci. USA* 1990, 87:2448-51; Renneisen *et al. J. Biol. Chem.* 1990, 265:16337-42).

EXAMPLE 20

Methods of Treatment using Antisense Molecules

When FGF-5 levels are prematurely downregulated by various antisense strategies, the FGF-5 expressing or over-expressing tumor may regress (see EXAMPLE 19). FGF-5 antisense oligonucleotides (EXAMPLE 19) can be used to disrupt cellular expression of FGF-5 proteins, which results in a decrease in FGF-5 expression or activity, such as by a factor of at least 20%, 50%, or 80%. The decrease in FGF-5 expression and/or activity may cause regression of FGF-5 expressing or non-expressing tumors.

The subject suffering from a disease in which FGF-5 is expressed or over-expressed can be treated with a therapeutically effective amount of FGF-5 antisense oligonucleotides. After the FGF-5 antisense has taken effect (FGF-5 levels are downregulated), for example after 24-48 hours, the subject can be monitored for regression of the tumor and/or lysis of the cells of the tumor.

Prophylactic Treatments

The treatments disclosed herein can also be used prophylactially, for example to inhibit or prevent progression to of a disease in which FGF-5 is expressed or over-expressed. Such administration is indicated where the treatment is shown to have utility for treatment or prevention of the disorder. The prophylactic use is indicated in conditions known or suspected of preceding progression to diseases associated with an undesired amount of FGF-5 expression. Such diseases may include tumors in which FGF-5 expression is elevated, such as RCC, breast cancer and prostate cancer.

EXAMPLE 21

Generation and Expression of FGF-5 Fusion Proteins

Methods for making fusion proteins are well known to those skilled in the art. For example U.S. Patent No. 6,057,133 to Bauer *et al.* (herein incorporated by reference) discloses methods for making fusion molecules composed of human interleukin-3 (hIL-3) variant or mutant proteins functionally joined to a second colony stimulating factor, cytokine, lymphokine, interleukin, hematopoietic growth factor or IL-3 variant. U.S. Patent No. 6,072,041 to Davis *et al.* (herein incorporated by reference) discloses the generation of fusion proteins comprising a single chain Fv molecule directed against a transcytotic receptor covalently linked to a therapeutic protein.

Similar methods can be used to generate fusion proteins comprising FGF-5 (or variants, mutants, polymorphisms, or fragments thereof) linked to other amino acid sequences. Linker

regions can be used to space the two portions of the protein from each other and to provide flexibility between them. The linker region is generally a polypeptide of between 1 and 500 amino acids in length, for example less than 30 amino acid in length. The linker joining the two molecules can be designed to (1) allow the two molecules to fold and act independently of each other, (2) not have a propensity for developing an ordered secondary structure which could interfere with the functional domains of the two proteins, (3) have minimal hydrophobic or charged characteristic which could interact with the functional protein domains and (4) provide steric separation of the two regions. Typically surface amino acids in flexible protein regions include Gly, Asn and Ser. Other neutral amino acids, such as Thr and Ala, can also be used in the linker sequence. Additional amino acids may also be included in the linker due to the addition of unique restriction sites in the linker sequence to facilitate construction of the fusions. Other moieties may also be included, as desired. These may include a binding region, such as avidin or an epitope, such as a polyhistadine tag, which may be useful for purification and processing of the fusion protein. In addition, detectable markers can be attached to the fusion protein, so that the traffic of the fusion protein through a body or cell may be monitored conveniently. Such markers may include radionuclides, enzymes, fluors, and the like.

Fusing of the nucleic acid sequences of FGF-5 (or variants, mutants, polymorphisms, or fragment thereof), with the nucleic acid sequence of another protein (or variants, mutants, polymorphisms, or fragment thereof), can be accomplished by the use of intermediate vectors. Alternatively, one gene can be cloned directly into a vector containing the other gene. Linkers and adapters can be used for joining the nucleic acid sequences, as well as replacing lost sequences, where a restriction site was internal to the region of interest. Genetic material (DNA) encoding one polypeptide, peptide linker, and the other polypeptide is inserted into a suitable expression vector which is used to transform prokaryotic or eukaryotic cells, for example bacteria, yeast, insect cells or mammalian cells (see EXAMPLE 8). The transformed organism is grown and the protein isolated by standard techniques, for example by using a detectable marker such as nickel-chelate affinity chromatography, if a polyhistadine tag is used. The resulting product is therefore a new protein, a fusion protein, which has FGF-5 joined by a linker region to a second protein. To confirm that the fusion protein was expressed, the purified protein is subjected to electrophoresis in SDS-polyacrylamide gels, and transferred onto nitrocellulose membrane filters using established methods. The protein products can be identified by Western blot analysis using antibodies directed against the individual components, i.e., polyhistadine tag and FGF-5 (see EXAMPLE 9).

EXAMPLE 28

FGF-5 Transgenic Plants and Animals

The creation of transgenic plants and animals which express FGF-5 can be made by techniques known in the art, for example those disclosed in U.S. Patent Nos. 5,574,206; 5,723,719; 5,175,383; 5,824,838; 5,811,633; 5,620,881; and 5,767,337, which are incorporated by reference. Methods for generating transgenic mice are described in *Gene Targeting*, Joyuner ed., Oxford University Press, 1995 and Watson *et al.*, *Recombinant DNA 2nd Ed.*, W.H. Freeman and Co., New York, 1992, Chapter 14.

EXAMPLE 29

Methods for Measuring an Immune Response

Several methods known to those skilled in the art can be used to monitor a modulation in an immune response in a subject. Although this example provides specific examples of assays which can be used for this purpose, other methods can be used to measure an immune response.

For example, modulations in an immune response, for example an increase or decrease in an immune response, can be measured by a observing a change in the activity or number of T-cells in the peripheral blood of a subject. In another embodiment, the immune response can be determined by measuring IFN-γ concentration, using the assays described in EXAMPLES 2 and 3. For example, when an immune response is increased, the concentration of IFN-γ may increase by a desired amount, for example by at least 50%, at least 75% or even at least 1000%. This immune response may reduced by a desired amount, for example by at least 50%, for example at least 75%, in the presence of pan-anti-class I MHC mAb (such as W6/32) or anti-HLA-A3 mAb (such as GAPA3).

The immune response can be modulated by increasing or decreasing the immune response. For example, FGF-5 or autologous CTLs specific to FGF-5, can be used provoke a CTL response against a tumor that is expressing or overexpressing FGF-5 in a subject to whom it is administered. In another embodiment, it is an amount of FGF-5 or autologous CTLs, specific to FGF-5, required to increase by more than a desired amount, for example by at least 50%, at least 75% or at least 1000% using the assay described in EXAMPLES 2 and 3.

Having illustrated and described the principles of methods of treating FGF-5 expressing or overexpressing tumors, it should be apparent to one skilled in the art that the disclosure can be modified in arrangement and detail without departing from such principles. In view of the many possible embodiments to which the principles of our disclosure may be applied, it should be

recognized that the illustrated embodiments are only examples of the disclosure and should not be taken as a limitation on the scope of the disclosure. Rather, the scope of the disclosure is in accord with the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A method of treating a subject having a neoplasm expressing fibroblast growth factor-5 (FGF-5) comprising:

a) modulating an immune response to FGF-5; or

b) modulating FGF-5 expression or activity.

2. The method of claim 1, wherein the neoplasm expressing FGF-5 is selected from the group consisting of a prostate carcinoma, a breast carcinoma, a bladder carcinoma, a pancreas carcinoma, and a renal cell carcinoma (RCC).

3. The method of claim 2, wherein the neoplasm is a RCC.

4. The method of claim 1, wherein the immune response is sufficient to stimulate a cytotoxic T cell response to a cell of the neoplasm.

5. The method of claim 4, wherein the cytotoxic T cell response is stimulated by administering a therapeutically effective amount of an agent that modulates an immune response.

6. The method of claim 5, wherein the agent that modulates an immune response is selected from the group consisting of an FGF-5 polypeptide and an immunoreactive sensitized T cell sensitized with FGF-5.

7. The method of claim 6, wherein the FGF-5 polypeptide that modulates an immune response comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence shown in SEQ ID NO: 4, 6, 8, 10, 12, 16, 18 or 19;

(b) amino acid sequences that differ from those specified in (a) by one or more conservative amino acid substitutions that retain the ability to modulate an immune response;

(c) fragments of the amino acid sequence of (a) or (b) that retain the ability to modulate an immune response; and

(d) amino acid sequences having at least 70% sequence identity to the sequences specified in (a), (b) and (c) that retain the ability to modulate an immune response.

8. The method of claim 7, wherein the FGF-5 polypeptide that modulates an immune response comprises an amino acid sequence selected from the group consisting of:

(a) the sequence shown in SEQ ID NO 8, 12, 18 or 19;

(b) an immunogenic fragment thereof that retains the ability to modulate an immune response; and

(c) a sequence having at least 70% sequence identity to (a) or (b) that retains the ability to modulate an immune response.

9. The method of claim 6, wherein administering the therapeutically effective amount of FGF-5 polypeptide comprises administering a nucleic acid encoding the FGF-5 polypeptide sufficient to stimulate a cytotoxic T cell response.

10. The method of claim 9, wherein administering the nucleic acid comprises administering an effective amount of a vector comprising a nucleic acid encoding the FGF-5 polypeptide sufficient to stimulate a cytotoxic T cell response to a cell of the neoplasm.

11. The method of claim 10, wherein the vector is a viral vector.

12. The method of claim 11, wherein the viral vector is a retroviral vector.

13. The method of claim 6, wherein administering the therapeutically effective amount of FGF-5 polypeptide comprises administering an effective amount of a host cell expressing a recombinant nucleic acid encoding FGF-5 sufficient to stimulate a cytotoxic T cell response to a cell of the neoplasm.

14. The method of claim 6, wherein administering the therapeutically effective amount of FGF-5 polypeptide comprises administering a purified FGF-5 polypeptide sufficient to stimulate a cytotoxic T cell response.

15. The method of claim 6, wherein the immunoreactive sensitized T cells sensitized with FGF-5 are autologous.

16. The method of claim 6, wherein the immunoreactive sensitized T cells sensitized with FGF-5 are heterologous.

17. The method of claim 1, wherein modulating FGF-5 expression or activity comprises decreasing FGF-5 expression or activity by administering a therapeutically effective amount of an agent that decreases FGF-5 expression or activity.

18. The method of claim 17, wherein the agent that decreases FGF-5 expression is an FGF-5 antisense molecule.

19. The method of claim 18, wherein the FGF-5 antisense molecule hybridizes to an RNA or a plus strand of an FGF-5 nucleic acid and decreases FGF-5 expression.

20. The method of claim 17, wherein the agent that decreases FGF-5 activity is an FGF-5 specific binding agent.

21. The method of claim 20 wherein the FGF-5 specific binding agent is capable of specifically binding to an FGF-5 polypeptide.

22. The method of claim 21, wherein the FGF-5 specific binding agent is selected from the group consisting of: polyclonal antibodies; monoclonal antibodies; and fragments of monoclonal antibodies.

23. The method of claim 1, wherein modulating FGF-5 expression or activity comprises increasing FGF-5 expression or activity by administering a therapeutically effective amount of an agent that increases FGF-5 expression or activity.

24. The method of claim 23, wherein the agent that increases FGF-5 expression is selected from the group consisting of an FGF-5 polypeptide and a nucleic acid encoding the FGF-5

polypeptide.

25. The method of claim 4, wherein the agent that modulates an immune response is therapeutically immunogenic in HLA-A3+ individuals.

26. The method of claim 25, further comprising the step of selecting HLA-A3+ individuals to whom to administer the agent that modulates an immune response.

27. A method of stimulating a cytotoxic T cell response against a RCC, comprising:

contacting the T cell with an effective amount of an FGF-5 polypeptide or a cell expressing the FGF-5 polypeptide sufficient to stimulate the T cell to react with a cell of the RCC.

28. The method of claim 5, wherein the agent that modulates an immune response is present in a pharmaceutically acceptable carrier.

29. The method of claim 17, wherein the agent that decreases FGF-5 expression or activity is present in a pharmaceutically acceptable carrier.

30. The method of claim 23, wherein the agent that increases FGF-5 expression or activity is present in a pharmaceutically acceptable carrier.

31. The method of claims 5, 17 and 23, further comprising administering one or more other anti-neoplastic compounds.

32. The method of claims 17 and 23, wherein the agent that decreases or increases FGF-5 expression or activity is present in a pharmaceutically acceptable carrier.

33. A method for detecting an enhanced susceptibility of a subject to a disease of abnormal FGF-5 expression, the method comprising detecting an increase in FGF-5 protein in the cell of the subject.

34. The method of claim 33 wherein the disease is selected from the group consisting of Hippel-Lindau disease, horseshoe kidneys, adult polycystic kidney disease, acquired renal cystic disease, a prostate carcinoma, a breast carcinoma, a bladder carcinoma, a pancreas carcinoma, and a RCC.

35. The method of claim 33 wherein the disease is RCC.

36. The method of claim 33, wherein the method comprises detecting FGF-5 protein in the cell of the subject, the method comprising:

incubating an FGF-5 specific binding agent with proteins of the cell under conditions such that the specific binding agent will specifically bind to a FGF-5 protein present in the cell to form a specific binding agent:FGF-5 protein complex; and

detecting an increase or decrease of specific binding agent:FGF-5 protein complexes, wherein an increase of the complexes relative to specific binding agent:FGF-5 protein complexes in a non-neoplastic cell indicates expression or overexpression of FGF-5, and an enhanced susceptibility of the subject to a disease of abnormal FGF-5 expression.

37. A method of lysing a cell of an FGF-5 expressing neoplasm in a subject, comprising sufficiently enhancing an immune response against FGF-5 in the subject, sufficient to induce regression of the neoplasm.

38. The method of claim 37, wherein the cell is characterized by increased expression of a FGF-5 protein compared to FGF-5 expression in a same tissue type that is non-neoplastic.

39. The method of claim 38, wherein enhancing the immune response comprises exposing the cell to a therapeutically effective amount of an FGF-5 polypeptide, sufficient to provoke an immune response against FGF-5.

40. The method of claim 38, wherein enhancing the immune response comprises administering a therapeutically effective amount of a nucleic acid which can express the FGF-5 polypeptide.

41. The method of claim 38, wherein enhancing the immune response comprises administering a therapeutically effective amount of immuno-reactive sensitized T-cells wherein the sensitized T cells are sensitized with FGF-5.

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(54) Title: FIBROBLAST GROWTH FACTOR-5 (FGF-5) IS A TUMOR ASSOCIATED T-CELL ANTIGEN

(57) Abstract: Disclosed herein are methods for treating tumors which express or over-express the tumor associated antigen (TAA) fibroblast growth factor 5 (FGF-5), including renal cell carcinoma (RCC) and carcinoma of the prostate and breast. Methods include modulating an immune response, such as increasing an immune response, or modulating FGF-5 expression or activity. The disclosure also includes methods of determining if a subject has an enhanced susceptibility to a disease associated with abnormal FGF-5 expression.

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Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln Trp Ser Pro Ser Gly Arg
     50                  55                  60

cgg acc ggc agc ctc tac tgc aga gtg ggc atc ggt ttc cat ctg cag   240
Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly Ile Gly Phe His Leu Gln
 65                  70                  75                  80

atc tac ccg gat ggc aaa gtc aat gga tcc cac gaa gcc aat atg tta   288
Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser His Glu Ala Asn Met Leu
                85                  90                  95

agt gtt ttg gaa ata ttt gct gtg tct cag ggg att gta gga ata cga   336
Ser Val Leu Glu Ile Phe Ala Val Ser Gln Gly Ile Val Gly Ile Arg
            100                 105                 110

gga gtt ttc agc aac aaa ttt tta gcg atg tca aaa aaa gga aaa ctc   384
Gly Val Phe Ser Asn Lys Phe Leu Ala Met Ser Lys Lys Gly Lys Leu
        115                 120                 125

cat gca agt gcc aag ttc aca gat gac tgc aag ttc agg gag cgt ttt   432
His Ala Ser Ala Lys Phe Thr Asp Asp Cys Lys Phe Arg Glu Arg Phe
    130                 135                 140

caa gaa aat agc tat aat acc tat gcc tca gca ata cat aga act gaa   480
Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser Ala Ile His Arg Thr Glu
145                 150                 155                 160

aaa aca ggg cgg gag tgg tat gtt gcc ctg aat aaa aga gga aaa gcc   528
Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu Asn Lys Arg Gly Lys Ala
                165                 170                 175

aaa cga ggg tgc agc ccc cgg gtt aaa ccc cag cat atc tct acc cat   576
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```
Lys Arg Gly Cys Ser Pro Arg Val Lys Pro Gln His Ile Ser Thr His
            180                 185                 190

ttt ctt cca aga ttc aag cag tcg gag cag cca gaa ctt tct ttc acg   624
Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln Pro Glu Leu Ser Phe Thr
        195                 200                 205

gtt act gtt cct gaa aag aaa aat cca cct agc cct atc aag tca aag   672
Val Thr Val Pro Glu Lys Lys Asn Pro Pro Ser Pro Ile Lys Ser Lys
    210                 215                 220

att ccc ctt tct gca cct cgg aaa aat acc aac tca gtg aaa tac aga   720
Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr Asn Ser Val Lys Tyr Arg
225                 230                 235                 240

ctc aag ttt cgc ttt gga taa                                     741
Leu Lys Phe Arg Phe Gly
                245
```


<210> 10
<211> 246
<212> PRT
<213> Homo sapiens

<400> 10

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Glu Lys Arg Leu Ala Pro Lys Gly Gln Pro Gly Pro Ala Ala Thr Asp
  1               5                  10                  15
Arg Asn Pro Ile Gly Ser Ser Ser Arg Gln Ser Ser Ser Ser Ala Met
            20                  25                  30
Ser Ser Ser Ser Ala Ser Ser Ser Pro Ala Ala Ser Leu Gly Ser Gln
        35                  40                  45
Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln Trp Ser Pro Ser Gly Arg
    50                  55                  60
Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly Ile Gly Phe His Leu Gln
65                  70                  75                  80
Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser His Glu Ala Asn Met Leu
                85                  90                  95
Ser Val Leu Glu Ile Phe Ala Val Ser Gln Gly Ile Val Gly Ile Arg
            100                 105                 110
Gly Val Phe Ser Asn Lys Phe Leu Ala Met Ser Lys Lys Gly Lys Leu
        115                 120                 125
His Ala Ser Ala Lys Phe Thr Asp Asp Cys Lys Phe Arg Glu Arg Phe
    130                 135                 140
Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser Ala Ile His Arg Thr Glu
145                 150                 155                 160
Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu Asn Lys Arg Gly Lys Ala
                165                 170                 175
Lys Arg Gly Cys Ser Pro Arg Val Lys Pro Gln His Ile Ser Thr His
            180                 185                 190
Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln Pro Glu Leu Ser Phe Thr
        195                 200                 205
Val Thr Val Pro Glu Lys Lys Asn Pro Pro Ser Pro Ile Lys Ser Lys
    210                 215                 220
Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr Asn Ser Val Lys Tyr Arg
225                 230                 235                 240
Leu Lys Phe Arg Phe Gly
                245
```

<210> 11
<211> 741
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(741)

<400> 11

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gag aag cgt ctc gcc ccc aaa ggg caa ccc gga ccc gct gcc act gat   48
Glu Lys Arg Leu Ala Pro Lys Gly Gln Pro Gly Pro Ala Ala Thr Asp
  1               5                  10                  15

agg aac cct aga ggc tcc agc agc aga cag agc agc agt agc gct atg   96
Arg Asn Pro Arg Gly Ser Ser Ser Arg Gln Ser Ser Ser Ser Ala Met
             20                  25                  30

tct tcc tct tct gcc tcc tcc tcc ccc gca gct tct ctg ggc agc caa   144
Ser Ser Ser Ser Ala Ser Ser Ser Pro Ala Ala Ser Leu Gly Ser Gln
         35                  40                  45

gga agt ggc ttg gag cag agc agt ttc cag tgg agc ccc tcg ggg cgc   192
Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln Trp Ser Pro Ser Gly Arg
     50                  55                  60

cgg acc ggc agc ctc tac tgc aga gtg ggc atc ggt ttc cat ctg cag   240
Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly Ile Gly Phe His Leu Gln
 65                  70                  75                  80

atc tac ccg gat ggc aaa gtc aat gga tcc cac gaa gcc aat atg tta   288
Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser His Glu Ala Asn Met Leu
                 85                  90                  95

agt gtt ttg gaa ata ttt gct gtg tct cag ggg att gta gga ata cga   336
Ser Val Leu Glu Ile Phe Ala Val Ser Gln Gly Ile Val Gly Ile Arg
            100                 105                 110

gga gtt ttc agc aac aaa ttt tta gcg atg tca aaa aaa gga aaa ctc   384
Gly Val Phe Ser Asn Lys Phe Leu Ala Met Ser Lys Lys Gly Lys Leu
        115                 120                 125

cat gca agt gcc aag ttc aca gat gac tgc aag ttc agg gag cgt ttt   432
His Ala Ser Ala Lys Phe Thr Asp Asp Cys Lys Phe Arg Glu Arg Phe
    130                 135                 140

caa gaa aat agc tat aat acc tat gcc tca gca ata cat aga act gaa   480
Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser Ala Ile His Arg Thr Glu
145                 150                 155                 160

aaa aca ggg cgg gag tgg tat gtg gcc ctg aat aaa aga gga aaa gcc   528
Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu Asn Lys Arg Gly Lys Ala
                165                 170                 175

aaa cga ggg tgc agc ccc cgg gtt aaa ccc cag cat atc tct acc cat   576
Lys Arg Gly Cys Ser Pro Arg Val Lys Pro Gln His Ile Ser Thr His
            180                 185                 190

ttt ctg cca aga ttc aag cag tcg gag cag cca gaa ctt tct ttc acg   624
Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln Pro Glu Leu Ser Phe Thr
        195                 200                 205
```

```
gtt act gtt cct gaa aag aaa aag cca cct agc cct atc aag cca aag   672
Val Thr Val Pro Glu Lys Lys Lys Pro Pro Ser Pro Ile Lys Pro Lys
    210                 215                 220

att ccc ctt tct gca cct cgg aaa aat acc aac tca gtg aaa tac aga   720
Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr Asn Ser Val Lys Tyr Arg
225                 230                 235                 240

ctc aag ttt cgc ttt gga taa                                     741
Leu Lys Phe Arg Phe Gly
                245
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<210> 12
<211> 246
<212> PRT
<213> Homo sapiens

<400> 12

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Glu Lys Arg Leu Ala Pro Lys Gly Gln Pro Gly Pro Ala Ala Thr Asp
  1               5                  10                  15
Arg Asn Pro Arg Gly Ser Ser Ser Arg Gln Ser Ser Ser Ser Ala Met
            20                  25                  30
Ser Ser Ser Ser Ala Ser Ser Ser Pro Ala Ala Ser Leu Gly Ser Gln
        35                  40                  45
Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln Trp Ser Pro Ser Gly Arg
    50                  55                  60
Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly Ile Gly Phe His Leu Gln
65                  70                  75                  80
Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser His Glu Ala Asn Met Leu
                85                  90                  95
Ser Val Leu Glu Ile Phe Ala Val Ser Gln Gly Ile Val Gly Ile Arg
            100                 105                 110
Gly Val Phe Ser Asn Lys Phe Leu Ala Met Ser Lys Lys Gly Lys Leu
        115                 120                 125
His Ala Ser Ala Lys Phe Thr Asp Asp Cys Lys Phe Arg Glu Arg Phe
    130                 135                 140
Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser Ala Ile His Arg Thr Glu
145                 150                 155                 160
Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu Asn Lys Arg Gly Lys Ala
                165                 170                 175
Lys Arg Gly Cys Ser Pro Arg Val Lys Pro Gln His Ile Ser Thr His
            180                 185                 190
Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln Pro Glu Leu Ser Phe Thr
        195                 200                 205
Val Thr Val Pro Glu Lys Lys Lys Pro Pro Ser Pro Ile Lys Pro Lys
    210                 215                 220
Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr Asn Ser Val Lys Tyr Arg
225                 230                 235                 240
Leu Lys Phe Arg Phe Gly
                245
```

<210> 13
<211> 920
<212> DNA
<213> Homo sapiens

<400> 13

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agagcccaga atcagcccta caagatgcac ttaggacccc cgcggctgga agaatgagct 120
tgtccttcct cctcctcctc ttcttcagcc acctgatcct cagcgcctgg gctcacgggg 180
agaagcgtct cgcccccaaa gggcaacccg gacccgctgc cactgatagg aaccctatag 240
gctccagcag cagacagagc agcagtagcg ctatgtcttc ctcttctgcc tcctcctccc 300
ccgcagcttc tctgggcagc caaggaagtg gcttggagca gagcagtttc cagtggagcc 360
cctcggggcg ccggaccggc agcctctact gcagagtggg catcggtttc catctgcaga 420
tctacccgga tggcaaagtc aatggatccc acgaagccaa tatgttaagt gttttggaaa 480
tatttgctgt gtctcagggg attgtaggaa tacgaggagt tttcagcaac aaatttttag 540
cgatgtcaaa aaaaggaaaa ctccatgcaa gtgccaagtt cacagatgac tgcaagttca 600
gggagcgttt tcaagaaaat agctataata cctatgcctc agcaatacat agaactgaaa 660
aaacagggcg ggagtggtat gttgccctga ataaaagagg aaaagccaaa cgagggtgca 720
gccccgggt taaaccccag catatctcta cccattttct tccaagattc aagcagtcgg 780
agcagccaga actttctttc acggttactg ttcctgaaaa gaaaaatcca cctagcccta 840
tcaagtcaaa gattcccctt tctgcacctc ggaaaaatac caactcagtg aaatacagac 900
tcaagtttcg ctttggataa 920

<210> 14
<211> 920
<212> DNA
<213> Homo sapiens

<400> 14
atgtccaccc ggtgcggcga ggcgggcagc gccagaggca cgcagccgca caggggctac 60
agagcccaga atcagcccta caagatgcac ttaggacccc cgcggctgga agaatgagct 120
tgtccttcct cctcctcctc ttcttcagcc acctgatcct cagcgcctgg gctcacgggg 180
agaagcgtct cgcccccaaa gggcaacccg gacccgctgc cactgatagg aaccctagag 240
gctccagcag cagacagagc agcagtagcg ctatgtcttc ctcttctgcc tcctcctccc 300
ccgcagcttc tctgggcagc caaggaagtg gcttggagca gagcagtttc cagtggagcc 360
cctcggggcg ccggaccggc agcctctact gcagagtggg catcggtttc catctgcaga 420
tctacccgga tggcaaagtc aatggatccc acgaagccaa tatgttaagt gttttggaaa 480
tatttgctgt gtctcagggg attgtaggaa tacgaggagt tttcagcaac aaatttttag 540
cgatgtcaaa aaaaggaaaa ctccatgcaa gtgccaagtt cacagatgac tgcaagttca 600
gggagcgttt tcaagaaaat agctataata cctatgcctc agcaatacat agaactgaaa 660
aaacagggcg ggagtggtat gtggccctga ataaaagagg aaaagccaaa cgagggtgca 720
gccccgggt taaaccccag catatctcta cccattttct gccaagattc aagcagtcgg 780
agcagccaga actttctttc acggttactg ttcctgaaaa gaaaaagcca cctagcccta 840
tcaagccaaa gattcccctt tctgcacctc ggaaaaatac caactcagtg aaatacagac 900
tcaagtttcg ctttggataa 920

<210> 15
<211> 1653
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (50)..(166)

<400> 15
cggacgcgtg ggctctctct tcccctctcc ccttctcttc cccgaggct atg tcc acc 58
Met Ser Thr
1

cgg tgc ggc gag gcg ggc agc gcc aga ggc acg cag ccg cac agg ggc 106
Arg Cys Gly Glu Ala Gly Ser Ala Arg Gly Thr Gln Pro His Arg Gly
5 10 15

tac aga gcc cag aat cag ccc tac aag atg cac tta gga ccc ccg cgg 154

Tyr Arg Ala Gln Asn Gln Pro Tyr Lys Met His Leu Gly Pro Pro Arg
20 25 30 35

ctg gaa gaa tga gcttgtcctt cctcctcctc ctcttcttca gccacctgat 206
Leu Glu Glu

cctcagcgcc tgggctcacg gggagaagcg tctcgccccc aaagggcaac ccggacccgc 266

tgccactgat aggaacccta gaggctccag cagcagacag agcagcagta gcgctatgtc 326

ttcctcttct gcctcctcct cccccgcagc ttctctgggc agccaaggaa gtggcttgga 386

gcagagcagt ttccagtgga gcccctcggg gcgccggacc ggcagcctct actgcagagt 446

gggcatcggt ttccatctgc agatctaccc ggatggcaaa gtcaatggat cccacgaagc 506

caatatgtta agtgttttgg aaatatttgc tgtgtctcag gggattgtag gaatacgagg 566

agttttcagc aacaaatttt tagcgatgtc aaaaaaagga aaactccatg caagtgccaa 626

gttcacagat gactgcaagt tcagggagcg ttttcaagaa aatagctata atacctatgc 686

ctcagcaata catagaactg aaaaaacagg gcgggagtgg tatgtggccc tgaataaaag 746

aggaaaagcc aaacgagggt gcagcccccg ggttaaaccc cagcatatct ctacccattt 806

tctgccaaga ttcaagcagt cggagcagcc agaactttct ttcacggtta ctgttcctga 866

aaagaaaaag ccacctagcc ctatcaagcc aaagattccc ctttctgcac ctcggaaaaa 926

taccaactca gtgaaataca gactcaagtt tcgctttgga taatattcct cttggccttg 986

tgagaaacca ttctttcccc tcaggagttt ctataggtgt cttcagagtt ctgaagaaaa 1046

attactggac acagcttcag ctatacttac actgtattga agtcacgtca tttgtttcag 1106

tgtgactgaa acaaaatgtt ttttgatagg aaggaaactg gaattctttg tactaataca 1166

gggagcacac tccttcagtt cagcaagaca taaagccttt tgctttatgc ttgagggata 1226

tttagaactt tgtattttcg gaaagttaaa taacagggac tacgtatttt tctgactttt 1286

acagattaac ctgaaagaac atacatgata cattttttatt tttggtttcc aaagaatatt 1346

ttgatgcaga taaaatattt tgttaacttt tgtttttttt tgtttgtttt cttaaaagta 1406

cctctgcatt gagcatattt tcttactttt attattttaa ttaatatgac ataagcaatc 1466

attttatgct gtttatgaat tataaatgtg tttatagctc atttgtaata tggaaatctt 1526

ttacattttt cctattcact gcacttttttt attgttttta tttctagcca tacctcagat 1586

aatatgttta gttttacatt ttaaaatgtt taaattctct ttcacagcaa aaaaaaaaaa 1646

aaaaaaa 1653

<210> 16
<211> 38
<212> PRT
<213> Homo sapiens

<400> 16
Met Ser Thr Arg Cys Gly Glu Ala Gly Ser Ala Arg Gly Thr Gln Pro
1 5 10 15
His Arg Gly Tyr Arg Ala Gln Asn Gln Pro Tyr Lys Met His Leu Gly
20 25 30
Pro Pro Arg Leu Glu Glu
35

<210> 17
<211> 1653
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (163)..(969)

<400> 17
cggacgcgtg ggctctctct tcccctctcc ccttctcttc cccgaggcta tgtccacccg 60

gtgcggcgag gcgggcagcg ccagaggcac gcagccgcac aggggctaca gagcccagaa 120

tcagccctac aagatgcact taggaccccc gcggctggaa ga atg agc ttg tcc 174
Met Ser Leu Ser
1

ttc ctc ctc ctc ctc ttc ttc agc cac ctg atc ctc agc gcc tgg gct 222
Phe Leu Leu Leu Leu Phe Phe Ser His Leu Ile Leu Ser Ala Trp Ala
5 10 15 20

cac ggg gag aag cgt ctc gcc ccc aaa ggg caa ccc gga ccc gct gcc 270
His Gly Glu Lys Arg Leu Ala Pro Lys Gly Gln Pro Gly Pro Ala Ala
25 30 35

act gat agg aac cct aga ggc tcc agc agc aga cag agc agc agt agc 318
Thr Asp Arg Asn Pro Arg Gly Ser Ser Ser Arg Gln Ser Ser Ser Ser
40 45 50

gct atg tct tcc tct tct gcc tcc tcc tcc ccc gca gct tct ctg ggc 366
Ala Met Ser Ser Ser Ser Ala Ser Ser Ser Pro Ala Ala Ser Leu Gly
55 60 65

agc caa gga agt ggc ttg gag cag agc agt ttc cag tgg agc ccc tcg 414
Ser Gln Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln Trp Ser Pro Ser
70 75 80

ggg cgc cgg acc ggc agc ctc tac tgc aga gtg ggc atc ggt ttc cat 462
Gly Arg Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly Ile Gly Phe His
85 90 95 100

ctg cag atc tac ccg gat ggc aaa gtc aat gga tcc cac gaa gcc aat 510
Leu Gln Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser His Glu Ala Asn
105 110 115

atg tta agt gtt ttg gaa ata ttt gct gtg tct cag ggg att gta gga 558
Met Leu Ser Val Leu Glu Ile Phe Ala Val Ser Gln Gly Ile Val Gly
120 125 130

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ata cga gga gtt ttc agc aac aaa ttt tta gcg atg tca aaa aaa gga   606
Ile Arg Gly Val Phe Ser Asn Lys Phe Leu Ala Met Ser Lys Lys Gly
        135                 140                 145

aaa ctc cat gca agt gcc aag ttc aca gat gac tgc aag ttc agg gag   654
Lys Leu His Ala Ser Ala Lys Phe Thr Asp Asp Cys Lys Phe Arg Glu
    150                 155                 160

cgt ttt caa gaa aat agc tat aat acc tat gcc tca gca ata cat aga   702
Arg Phe Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser Ala Ile His Arg
165                 170                 175                 180

act gaa aaa aca ggg cgg gag tgg tat gtg gcc ctg aat aaa aga gga   750
Thr Glu Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu Asn Lys Arg Gly
                185                 190                 195

aaa gcc aaa cga ggg tgc agc ccc cgg gtt aaa ccc cag cat atc tct   798
Lys Ala Lys Arg Gly Cys Ser Pro Arg Val Lys Pro Gln His Ile Ser
            200                 205                 210

acc cat ttt ctg cca aga ttc aag cag tcg gag cag cca gaa ctt tct   846
Thr His Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln Pro Glu Leu Ser
        215                 220                 225

ttc acg gtt act gtt cct gaa aag aaa aag cca cct agc cct atc aag   894
Phe Thr Val Thr Val Pro Glu Lys Lys Lys Pro Pro Ser Pro Ile Lys
    230                 235                 240

cca aag att ccc ctt tct gca cct cgg aaa aat acc aac tca gtg aaa   942
Pro Lys Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr Asn Ser Val Lys
245                 250                 255                 260

tac aga ctc aag ttt cgc ttt gga taa tattcctctt ggccttgtga        989
Tyr Arg Leu Lys Phe Arg Phe Gly
                265
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```
gaaaccattc tttcccctca ggagtttcta taggtgtctt cagagttctg aagaaaaatt 1049

actggacaca gcttcagcta tacttacact gtattgaagt cacgtcattt gtttcagtgt 1109

gactgaaaca aaatgttttt tgataggaag gaaactggaa ttctttgtac taatacaggg 1169

agcacactcc ttcagttcag caagacataa agccttttgc tttatgcttg agggatattt 1229

agaactttgt attttcggaa agttaaataa cagggactac gtatttttct gacttttaca 1289

gattaacctg aaagaacata catgatacat ttttatttct ggtttccaaa gaatattttg 1349

atgcagataa aatattttgt taacttttgt tttttttttgt ttgttttctt aaaagtacct 1409

ctgcattgag catattttct tacttttatt attttaatta atatgacata agcaatcatt 1469

ttatgctgtt tatgaattat aaatgtgttt atagctcatt tgtaatatgg aaatctttta 1529

catttttcct attcactgca ctttttttatt gtttttattt ctagccatac ctcagataat 1589

atgtttagtt ttacatttta aaatgtttaa attctctttc acagcaaaaa aaaaaaaaaa 1649

aaaa                                                              1653
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<210> 18
<211> 268
<212> PRT
<213> Homo sapiens

<400> 18
Met Ser Leu Ser Phe Leu Leu Leu Leu Phe Phe Ser His Leu Ile Leu
1 5 10 15
Ser Ala Trp Ala His Gly Glu Lys Arg Leu Ala Pro Lys Gly Gln Pro
20 25 30
Gly Pro Ala Ala Thr Asp Arg Asn Pro Arg Gly Ser Ser Ser Arg Gln
35 40 45
Ser Ser Ser Ser Ala Met Ser Ser Ser Ser Ala Ser Ser Ser Pro Ala
50 55 60
Ala Ser Leu Gly Ser Gln Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln
65 70 75 80
Trp Ser Pro Ser Gly Arg Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly
85 90 95
Ile Gly Phe His Leu Gln Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser
100 105 110
His Glu Ala Asn Met Leu Ser Val Leu Glu Ile Phe Ala Val Ser Gln
115 120 125
Gly Ile Val Gly Ile Arg Gly Val Phe Ser Asn Lys Phe Leu Ala Met
130 135 140
Ser Lys Lys Gly Lys Leu His Ala Ser Ala Lys Phe Thr Asp Asp Cys
145 150 155 160
Lys Phe Arg Glu Arg Phe Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser
165 170 175
Ala Ile His Arg Thr Glu Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu
180 185 190
Asn Lys Arg Gly Lys Ala Lys Arg Gly Cys Ser Pro Arg Val Lys Pro
195 200 205
Gln His Ile Ser Thr His Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln
210 215 220
Pro Glu Leu Ser Phe Thr Val Thr Val Pro Glu Lys Lys Lys Pro Pro
225 230 235 240
Ser Pro Ile Lys Pro Lys Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr
245 250 255
Asn Ser Val Lys Tyr Arg Leu Lys Phe Arg Phe Gly
260 265

<210> 19
<211> 60
<212> PRT
<213> Homo sapiens

<400> 19
Lys Phe Arg Glu Arg Phe Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser
1 5 10 15

Ala Ile His Arg Thr Glu Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu
20 25 30

Asn Lys Arg Gly Lys Ala Lys Arg Gly Cys Ser Pro Arg Val Lys Pro
35 40 45

Gln His Ile Ser Thr His Phe Leu Pro Arg Phe Lys
50 55 60

<210> 20
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Forward Primer

<400> 20
cttcttcagc cacctgatcc tc 22

<210> 21
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Forward Primer

<400> 21
tgcagagtgg gcatcggttt c 21

<210> 22
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Reverse Primer

<400> 22
tatgctcaat gcagaggtac 20

<210> 23
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Reverse Primer

<400> 23
cgtagtccct gttatttaac 20

<210> 24
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer that can be used to clone HLA-A3

<400> 24

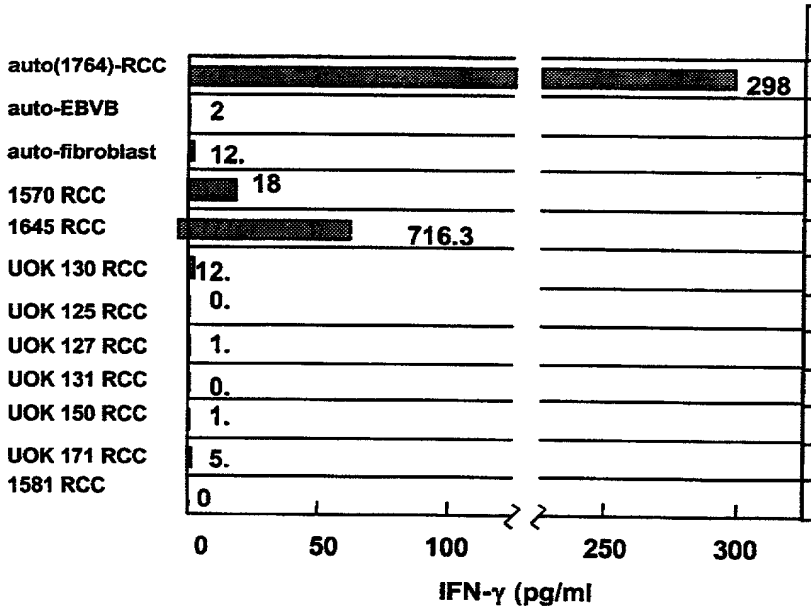
ttggggaggg agcacaggtc agcgtgggaa g 31

<210> 25
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer that can be used to clone HLA-A3.

<400> 25
ggactcagaa tctccccaga cgccgag 27

Figure 1



HLA-	HLA-	HLA-
1,	8,	5
2,	15,	10
1,	7,	6,
2,	7,	1,
2,	40,	2,6
1,	15,	6,1
2,	44	4
2,	40,	3,
23,6	64,	2,
1,	8,	7,

Figure 2A

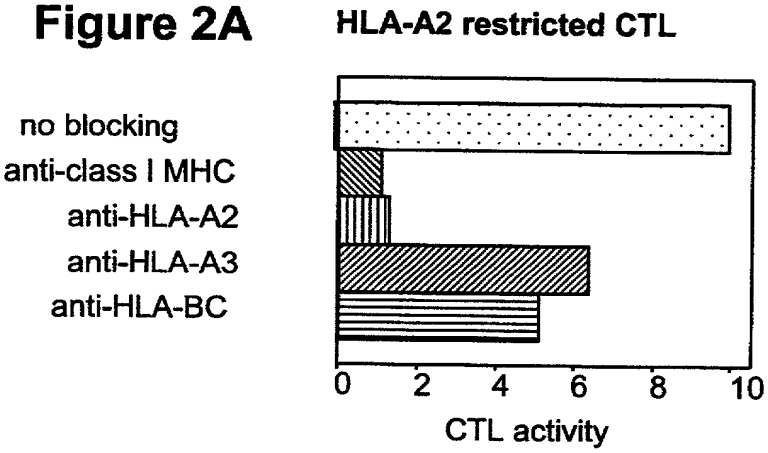


Figure 2B

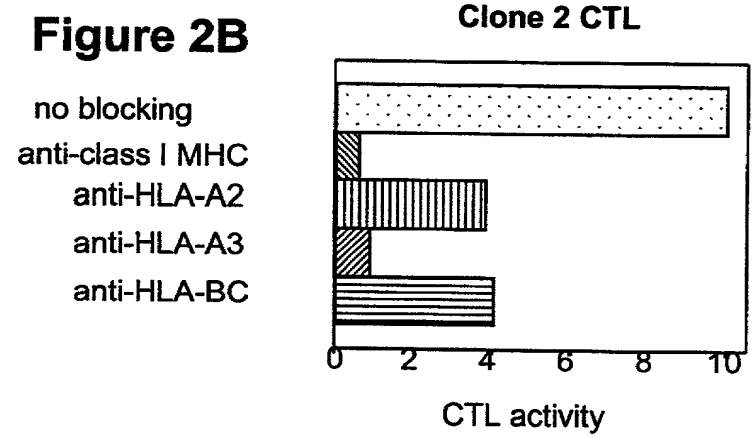


Figure 3A

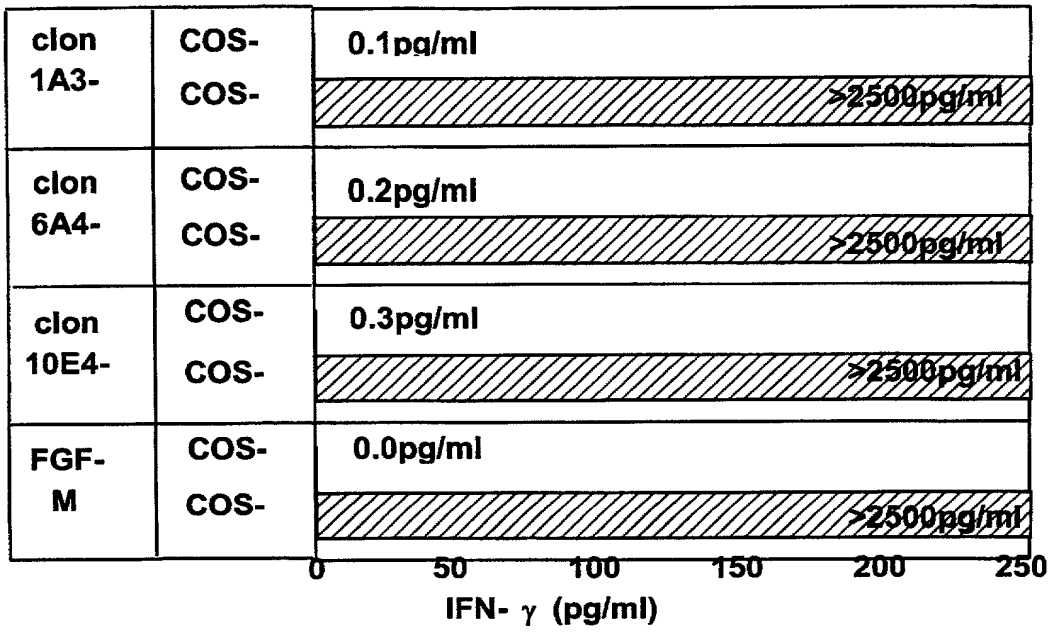


Figure 3B

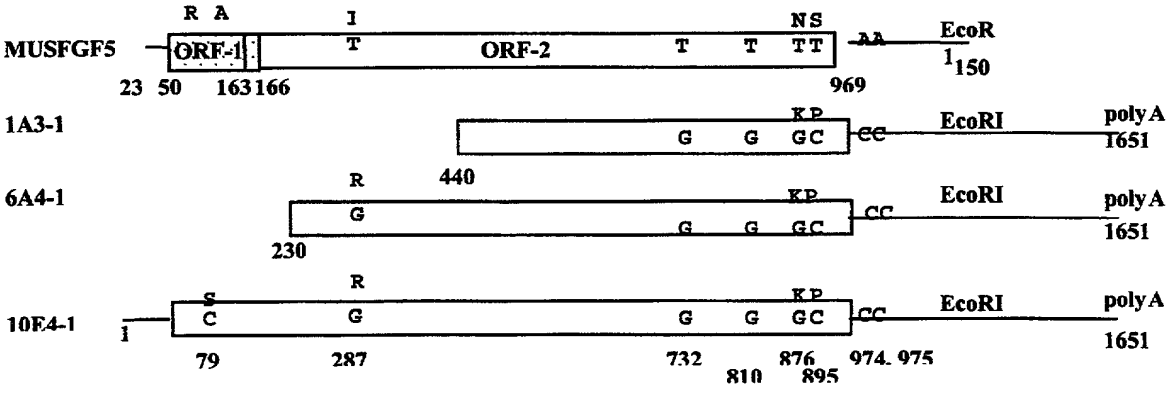


Figure 4

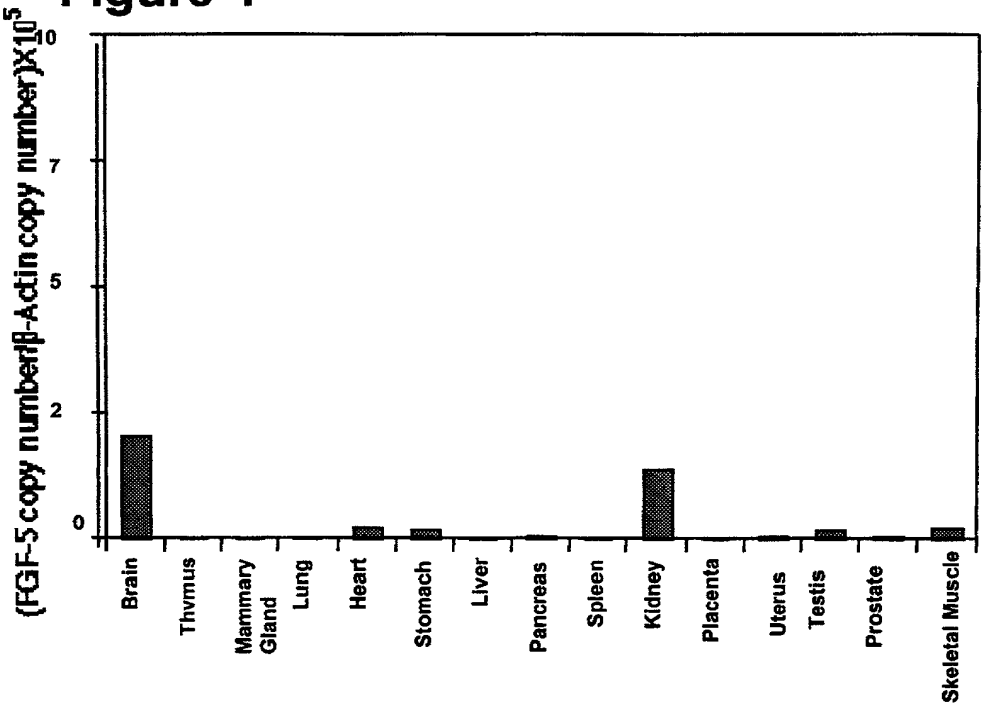


Figure 5

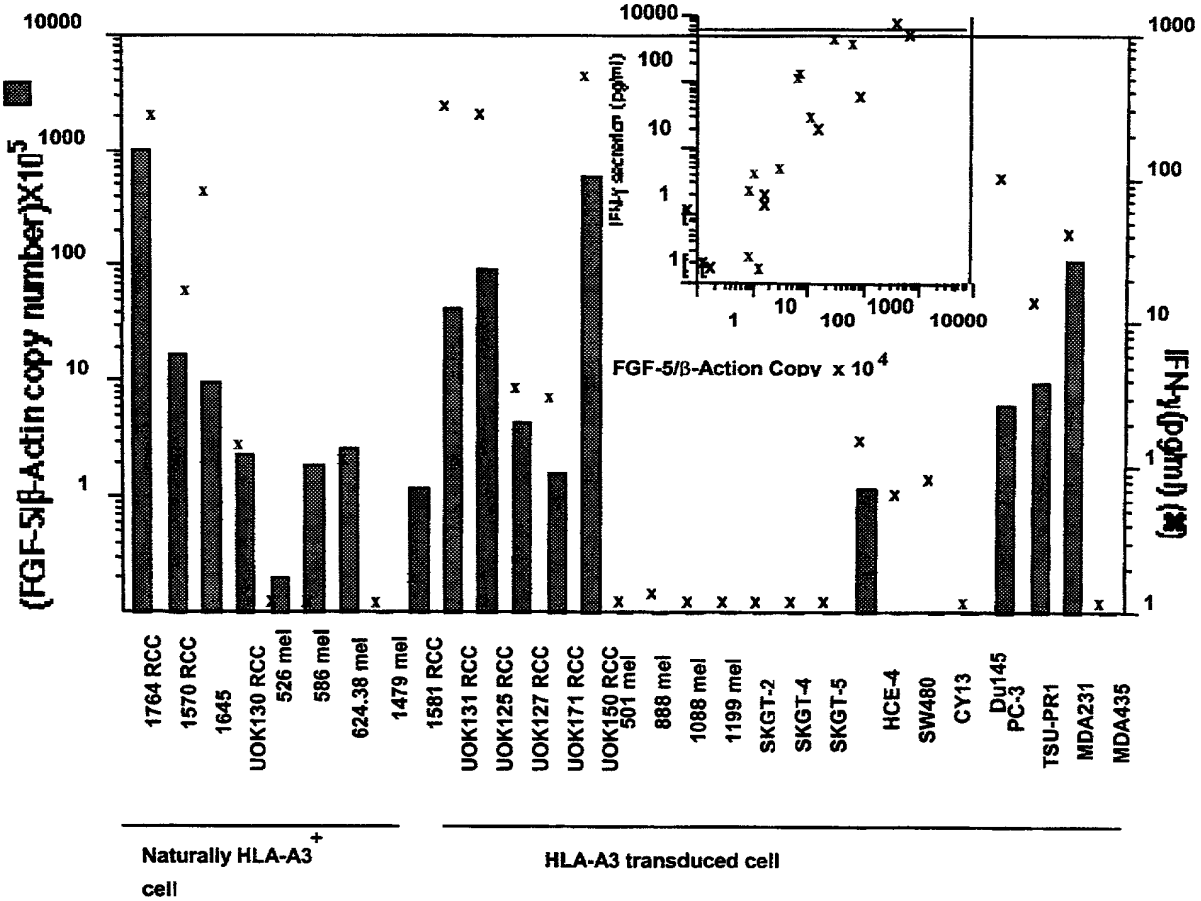


Figure 6A

```
ORF-1.....METSerThrArgCysGly                    6
CCTCTCCCCTTCTCTTCCCCGAGGCTATGTCCACCCGGTGCGGC   44

GluAlaGlyGlnSerArgGlyThrGlnProHisArgGlyTyrArg  21
GAGGCGGGCCAGAGCAGAGGCACGCAGCCGCACAGGGGCTACAGA  89

AlaGlnAsnGlnProTyrLysMetHisLeuGlyProProArgLeu  36
GCCCAGAATCAGCCCTACAAGATGCACTTAGGACCCCCGCGGCTG 134
ORF-2.....

GluGluEND                                      38
GAAGAATGAGCTTGTCCTTCCTCCTCCTCCTCTTCTTCAGCCAC  178
.....METSerLeuSerPheLeuLeuLeuLeuPhePheSerHis   13

CTGATCCTCAGCGCCTGGGCTCACGGGGAGAAGCGTCTCGCCCCC 223
LeuIleLeuSerAlaTrpAlaHisGlyGluLysArgLeuAlaPro  28

AAAGGGCAACCCGGACCCGCTGCCACTGATAGGAACCCTAGAGGC 268
LysGlyGlnProGlyProAlaAlaThrAspArgAsnProArgGly  43
```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10/089485

Figure 6B

```
TCCAGCAGCAGACAGAGCAGCAGTAGCGCTATGTCTTCCTCTTCT      313
SerSerSerArgGlnSerSerSerSerAlaMetSerSerSerSer       58

GCCTCCTCCTCCCCCGCAGCTTCTCTGGGCAGCCAAGGAAGTGGC     358
AlaSerSerSerProAlaAlaSerLeuGlySerGlnGlySerGly       73

TTGGAGCAGAGCAGTTTCCAGTGGAGCCTCGGGGCGCGGACCGGC     403
LeuGluGlnSerSerPheGlnTrpSerLeuGlyAlaArgThrGly       88

AGCCTCTACTGCAGAGTGGGCATCGGTTTCCATCTGCAGATCTAC     448
SerLeuTyrCysArgValGlyIleGlyPheHisLeuGlnIleTyr      103

CCGGATGGCAAAGTCAATGGATCCCACGAAGCCAATATGTTAAGT     493
ProAspGlyLysValAsnGlySerHisGluAlaAsnMetLeuSer      118

GTTTTGGAAATATTTGCTGTGTCTCAGGGGATTGTAGGAATACGA     538
ValLeuGluIlePheAlaValSerGlnGlyIleValGlyIleArg      133
```

Figure 6C

```
GGAGTTTTCAGCAACAAATTTTTAGCGATGTCAAAAAAAGGAAAA      583
GlyValPheSerAsnLysPheLeuAlaMetSerLysLysGlyLys      148

CTCCATGCAAGTGCCAAGTTCACAGATGACTGCAAGTTCAGGGAG      628
LeuHisAlaSerAlaLysPheThrAspAspCysLysPheArgGlu      163

CGTTTTCAAGAAAATAGCTATAATACCTATGCCTCAGCAATACAT      673
ArgPheGlnGluAsnSerTyrAsnThrTyrAlaSerAlaIleHis      178

AGAACTGAAAAAACAGGGCGGGAGTGGTATGTTGCCCTGAATAAA      718
ArgThrGluLysThrGlyArgGluTrpTyrValAlaLeuAsnLys      193

AGAGGAAAAGCCAAACGAGGGTGCAGCCCCCGGGTTAAACCCCAG      763
ArgGlyLysAlaLysArgGlyCysSerProArgValLysProGln      208

CATATCTCTACCCATTTTCTTCCAAGATTCAAGCAGTCGGAGCAG      808
HisIleSerThrHisPheLeuProArgPheLysGlnSerGluGln      223
```

Figure 6D

```
CCAGAACTTTCTTTCACGGTTACTGTTCCTGAAAAGAAAAATCCA      853
ProGluLeuSerPheThrValThrValProGluLysLysAsnPro      238

CCTAGCCCTATCAAGTCAAAGATTCCCCTTTCTGCACCTCGGAAA      898
ProSerProIleLysSerLysIleProLeuSerAlaProArgLys      253

AATACCAACTCAGTGAAATACAGACTCAAGTTTCGCTTTGGATAA      943
AsnThrAsnSerValLysTyrArgLeuLysPheArgPheGlyEND      267

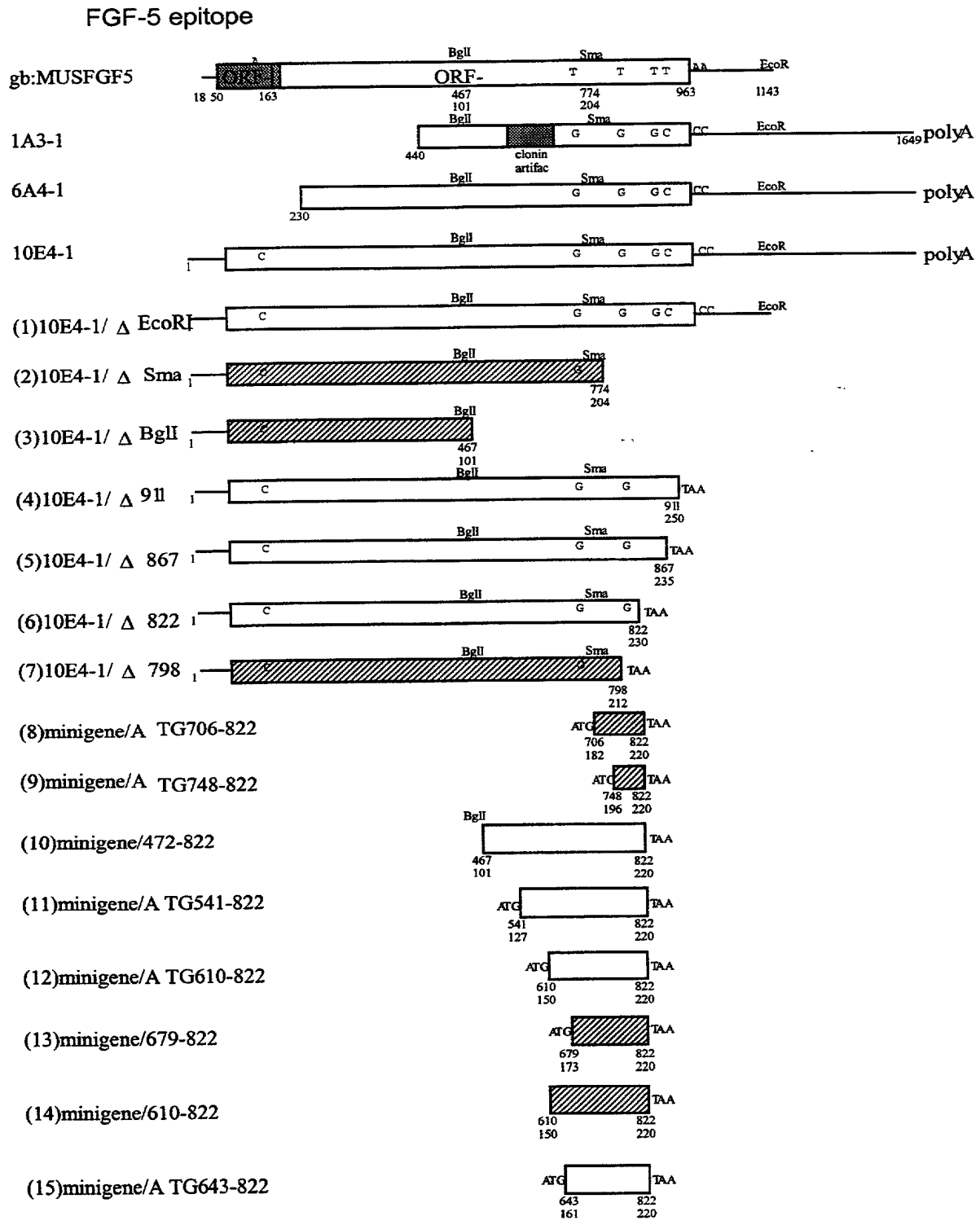
TATTAATCTTGGCCTTGTGAGAAACCATTCTTTCCCCTCAGGAGT      988

TTCTATAGGTGTCTTCAGAGTTCTGAAGAAAAATTACTGGACACA     1033

GCTTCAGCTATACTTACACTGTATTGAAGTCACGTCATTTGTTTC     1078

AGTGTGACTGAAACAAAATGTTTTTTGATAGGAAGGAAACTG        1120
```

Figure 7


FGF-5 epitope
gb:MUSFGF5
ORF-
BglI
Sma
EcoR
1143
1A3-1
clonin artifac
1649polyA
6A4-1
polyA
10E4-1
(1)10E4-1/ Δ EcoRI
(2)10E4-1/ Δ Sma
(3)10E4-1/ Δ BglI
(4)10E4-1/ Δ 9 1l
(5)10E4-1/ Δ 867
(6)10E4-1/ Δ 822
(7)10E4-1/ Δ 798
(8)minigene/A TG706-822
(9)minigene/A TG748-822
(10)minigene/472-822
(11)minigene/A TG541-822
(12)minigene/A TG610-822
(13)minigene/679-822
(14)minigene/610-822
(15)minigene/A TG643-822

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled, the specification of which

☐ is attached hereto.

☐ was filed on _____ as United States Application No. _____.

☒ was filed on 29 September 2000 as International Application No. PCT/US00/26689.

☐ and was amended on _____ (if applicable).

☐ with amendments through _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56. If this is a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, I further acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of an PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)	Country	Filing Date	Priority Claimed
			☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

60/157,103	2 October 1999
(Application No.)	(Filing Date)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the

subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

(Application No.)	(Filing Date)	(Status: patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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